AD_____

Award Number: DAMD17-01-1-0784

TITLE: 2001 Congress on In Vitro Biology

PRINCIPAL INVESTIGATOR: John W. Harbell, Ph.D.

CONTRACTING ORGANIZATION: Society for In Vitro Biology
Largo, Maryland 20774

REPORT DATE: July 2002

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Final Proceedings (1 Jul 01 - 31 Dec 01)

4. TITLE AND SUBTITLE
2001 Congress on In Vitro Biology

5. FUNDING NUMBERS
DAMD17-01-1-0784

6. AUTHOR(S):
John W. Harbell, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Society for In Vitro Biology
Largo, Maryland 20774

Email: jharbell@iivs.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

none provided

14. SUBJECT TERMS
In Vitro Biology

15. NUMBER OF PAGES
102

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

IN VITRO

VOLUME 37 NUMBER 3, PART II MARCH 2001

CELLULAR & DEVELOPMENTAL BIOLOGY

PROGRAM ISSUE

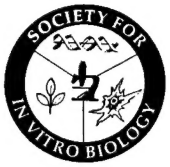
2001

Congress on In Vitro Biology

June 16 - 20, 2001
St. Louis, Missouri

The Regal Riverfront Hotel

PROGRAM ISSUE



Journal of the Society for In Vitro Biology

Live-Cell Microscopy Environmental Control

Standardized Techniques from Bioptechs

After rigorous preparation, your cells need an environment that is both conducive to their viability and compatible with all modes of microscopy.

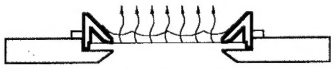
Bioptechs has developed a variety of live-cell microscopy environmental control products which provide far superior optical and thermal performance as compared to traditional methods. See how accurately, easily and inexpensively this technology can solve your live-cell micro-observation needs.

Open System — Confocal Compatible!

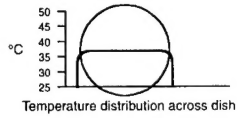
ΔTC3 Live-Cell Culture Dish

First-Surface Thermal Transfer Technique

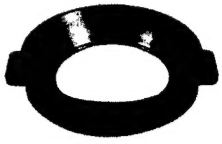
Ensure the viability of your cells & accuracy of your data. Take advantage of the first-surface thermal transfer technology that offers convenience, economy, versatility & optical superiority over traditional live-cell microscopy environmental control methods!

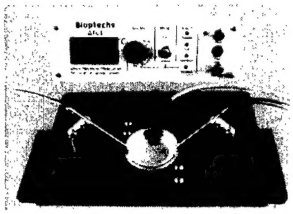


Coverslip bottomed, self-heating glass surface, low dead-volume dish



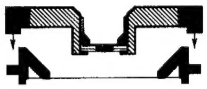
Temperature distribution across dish



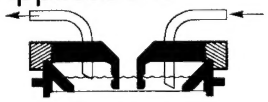


Think of it as a precision, miniature, coverglass bottomed hot tub for your cells!

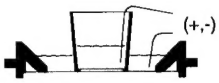
Other ΔTC3 Dish Applications



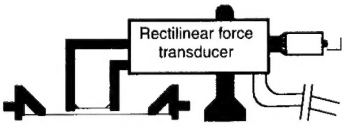
Brain slice micro-observation chamber



Tissue slice chamber



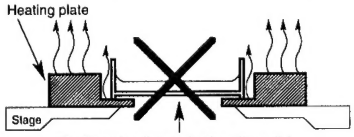
Artificial membrane insert micro-observation chamber



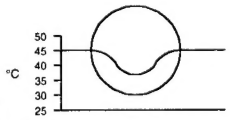
Isometric muscle slice force transduction

Obsolete Culture Dish Heating

Non-uniform heat distribution, not high N.A. compatible, slow temperature transfer, unstable in Z axis and subject to surface evaporation induced temperature changes.



Traditional ordinary plastic culture dish

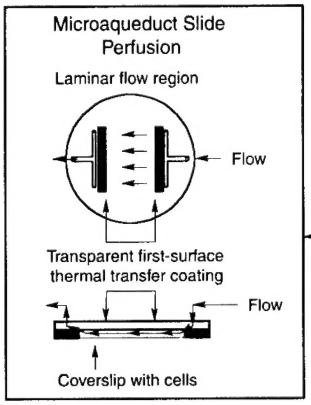


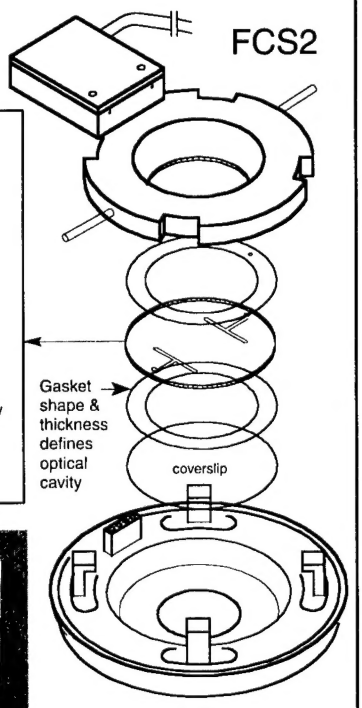
Temperature distribution across dish

Closed System

Micro-Observation Environment

FCS2





The FCS2 Closed System Chamber includes a two-channel controller which regulates the temperature of the microaqueduct slide and chamber plus a separate controller for the objective heater for use with high N.A. objectives.

Features include:

- Uniform temperature transfer direct to cells.
- Temperature stabilization or recovery in seconds.
- Compatible with all modes of light microscopy.
- Laminar flow perfusion w/low cell surface shear.
- Complete volume exchange as short as 1.0 sec.
- User definable volume & flow path.
- No need for an air-curtain.

Get the most out of your high quality CCD camera with QED Imaging. An intuitive, full function, quantitative, time-lapse acquisition package that works inside NIH Image, Photoshop or other IP programs that support Photoshop type plug-ins.

Bioptechs, Inc.

3560 Beck Rd., Butler, PA 16002 Web, www.bioptechs.com

Toll-Free 877 LIVE-CELL V724-282-7145 F724-282-0745 Email info@bioptechs.com

IN VITRO
CELLULAR & DEVELOMENTAL BIOLOGY

VOLUME 37 NUMBER 3 PART II
ISSN 1071-2690
MARCH 2001

Program Schedule

Saturday, June 16

Sunday, June 17

Monday, June 18

Tuesday, June 19

Wednesday, June 20

Abstracts

2001 CONGRESS ON IN VITRO BIOLOGY
ORGANIZING / PROGRAM PLANNING COMMITTEE

IN VITRO
ANIMAL
CELLULAR & DEVELOPMENTAL BIOLOGY

Journal of the
Society for
In Vitro Biology

IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY (ISSN 1071-2690) is published monthly, except bi-monthly with the July/August and November/December issues, by the Society for In Vitro Biology (SIVB), 9315 Largo Drive West, Suite 255, Largo, MD 20774. *IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY* is devoted to the advancement and dissemination of basic and applied knowledge concerning the in vitro cultivation of cells, tissues, organs, or tumors from multicellular organisms or plants. Non-member subscription rate: $290.00 per volume ($325.00 outside North America via expedited air service), payable in advance in U.S.A. funds drawn on a U.S.A. bank. If not a U.S.A. bank, add $25.00 bank clearance fee. Claims of nonreceipt should be made within six months of publication. Back issues may be purchased at $30.00 per issue. SIVB members receive *IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY* ($30.00) as part of annual dues. Correspondence relating to subscriptions, nonreceipt of journals, back issues, advertising, and late or lost proofs should be directed to the Society for In Vitro Biology, 9315 Largo Drive West, Suite 255, Largo, MD 20774 (301) 324-5054. Periodicals postage paid at Upper Marlboro, MD, and additional mailing offices.

Postmaster: Send changes to *IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY*, 9315 Largo Drive West, Suite 255, Largo, MD 20774.

Made in the United States of America

In Vitro Cellular & Developmental Biology — Plant

A journal of the Society for In Vitro Biology published by CABI Publishing
Volume 37 2001. First published 1965. ISSN 1054–5476

Aims and scope

In Vitro Cellular & Developmental Biology– Plant publishes peer-reviewed original research and reviews concerned with the latest developments and state-of-the-art research in plant cell and tissue culture and biotechnology from around the globe. Four issues cover cellular, molecular and developmental biology research using *in vitro* grown or maintained organs, tissues or cells derived from plants. Two special IAPTC&B issues deal with plant tissue culture, and molecular and cellular aspects of plant biotechnology. The IAPTC&B and SIVB maintain completely separate and independent International Editorial Review boards for their issues. From the start of the 2000 volume *In Vitro Cellular and Developmental Biology — Plant* will be available in print and on the Internet in Acrobat PDF and HTML formats.

Topics covered by the journal include:

- biotechnology/genetic transformation
- developmental biology/morphogenesis
- micropropagation
- functional genomics
- molecular farming
- metabolic engineering
- plant physiology
- cell biology
- somatic cell genetics
- secondary metabolism

Saturday

7:00	Registration-Marble Area
8:00 - 11:00	Risk Communication: Scientists and the Public: Handling Controversial Issues General Workshop — Field Educational Outreach Program K-12 for Teachers In Vitro Techniques for the Classroom: Bringing DNA to the Classroom Education Workshop — Clark
8:00 – 12:00	SIVB Board of Directors Meeting — Board Room
11:00 - 12:00	In Vitro Research by High School Students Student Symposium — Lewis West
12:00- 1:00	2001 Program Planning Committee Meeting — Laclede
12:00- 4:00	Biotechnology Risk Communication Training – In-depth Training Which Meets Your Needs General Workshop — Field Advanced Tissue Culture Workshop: Assays for Cell Viability Joint Vertebrate/Invertebrate/Toxicology Workshop — Lewis East
1:00 – 4:00	Educational Outreach Program K-12 for Teachers In Vitro Techniques for the Classroom: Plant Culture Tissue in the Classroom Educational Workshop — Clark
4:00 – 7:00	Monsanto Tour — Monsanto
Poster Information	SUN. JUNE 17 10:00 – 9:00 pm; MON. JUNE 18 10:00 – 9:00 pm; TUES. JUNE 19 10:00 – 2:30 pm POSTER SESSION Posters mounted Saturday, June 16 from 3:00 – 6:00 pm. Posters must be removed from Exhibit Hall by 3:00 pm, June 19. Authors will be present at their posters the following days and times: Sat. June 16 All Authors 7:30 – 8:30pm; Sun. June 17 Even Authors 2:15 – 2:45pm; Mon. June 18 Odd Authors 2:15 – 2:45pm; Tues. June 19 All Authors 2:15 – 2:30pm **NEW INTERACTIVE POSTER SESSIONS** Sunday 6/17 through Tuesday 6/19 – Moderated poster sessions are being presented. Specific sessions are listed on this schedule chart and in the program on their presented days and times.
Evening Events	2001 Congress Opening Reception 7:00 pm — Exhibit Hall

Sunday

7:00– 8:00	Publications Committee — Lewis East Cell Culture Standardization Committee Meeting — Lewis West
8:00 - 10:00	Artificial Chromosomes Joint Vertebrate/Plant Symposium — Jefferson F Development and Application of Cell Models for Pre-clinical Research Vertebrate/Toxicology Contributed Paper Session — Jefferson D
10:00	Coffee Break
10:00 – 3:00 Exhibits and Posters, 10:30 - 12:30	Beyond the Genome: Functional Genomics Joint Vertebrate/Invertebrate/Toxicology Symposium — Lewis Deployment of Transgenic Trees Plant Symposium — Jefferson D & E
10:00 – 3:00 Exhibits and Posters, 12:30 - 1:30	The Proteomics Approach to Understanding a Biological System Joint Vertebrate/Invertebrate/Toxicology Workshop — Clark
10:00 – 3:00 Exhibits and Posters, 1:15 – 2:15	*INTERACTIVE POSTER SESSIONS* — EXHIBIT HALL Secondary Metabolism — Plant Disease Resistance — Plant In Vitro Toxicology — Vertebrate/Toxicology
3:00 – 4:30	PLENARY SESSION — Missouri/Illinois "Opportunities and Challenges in Plant Biotechnology to Benefit Health and Sustainability"
5:30	AN EVENING AT THE MISSOURI BOTANICAL GARDENS Buses depart for Botanical Gardens
6:00 – 7:00	Plenary Reception — Missouri Botanical Gardens
7:00 – 9:00	Dinner — Missouri Botanical Gardens

Monday			Tuesday		
7:00		Registration-Marble Area			
8:00-10:00		Insect Hormones and Applications for Pest Control Invertebrate Symposium — Field Reproductive Health: Assessing the Role of Endocrine Disrupters In Vitro Vertebrate Symposium — Laclede Applications of In Vitro Culture for Habitat Restoration Plant Symposium — Jefferson B & C Dicot Transformation Plant Contributed Paper Session — Mississippi	8:00-10:00		Novel Breeding Strategies Plant Symposium — Missouri Understanding the Basis of Stem Cell Pluripotency/ Plant Cell Totipotiency Joint Plant/Vertebrate/Invertebrate Symposium — Jefferson B & C Frontiers in Low-temperature Preservation of Cells and Tissues Joint Vertebrate/Toxicology Workshop — Laclede
10:00		Coffee Break			
10:00-3:00 Exhibits and Posters	10:30-12:30	ABC Transporters: Their Role in Multi-drug Resistance, Bioavailability, and Drug-drug Interactions Toxicology Symposium — Laclede Nutraceuticals/Edible Vaccines Plant Symposium — Jefferson B & C	10:00 – 2:30 Exhibits and Posters	10:30-12:30	Primary Human Cell Cultures or Immortalized Cells: Models for Use in Human Toxicology and Disease Studies Vertebrate/Toxicology Workshop — Laclede Rooting of Micropropagated Plants Plant Symposium — Missouri Monocot Transformation Plant Contributed Paper Session — Jefferson B & C
	12:30-1:15	Exhibitors/SIVB Reception — Exhibit Hall			
	1:15 -2:15	*INTERACTIVE POSTER SESSIONS* — EXHIBIT HALL Dicot Transformation — Plant Part I: Evaluation of Cryopreservation Techniques Part II: Human Epidermal Keratinocytes — Vertebrate/Toxicology	1:15 – 2:15		*INTERACTIVE POSTER SESSIONS* — EXHIBIT HALL Monocot Transformation — Plant Ocular Models — Vertebrate/Toxicology
			2:15 – 2:30		Poster Session — Exhibit Hall All Poster Authors in Attendance
2:15 – 2:45		Poster Session — Exhibit Hall Odd Poster Authors in Attendance	3:00 – 4:00		Invertebrate Cells Invertebrate Contributed Paper Session — Jefferson C
3:15 – 5:45		Regulatory Affairs/Public Acceptance Plant Symposium — Jefferson B & C	3:00 – 5:00		Inducible Gene Systems Plant Symposium — Missouri Toxicological Applications of Commercially Available Epithelial Models Toxicology Symposia — Laclede
3:30 – 5:30		The Importance of Controls for In Vitro Biology Vertebrate/Toxicology Workshop — Laclede			
Evening Events		Plant Section Business Meeting — 6:00 – 7:00 Plant Section Social — 7:00 – 9:00 Mississippi Vertebrate/Cellular Toxicology Business Meeting and Social — 7:30 – 9:30 Laclede	Evening Events		Reception / Silent Auction 7:00 pm – 8:00 pm — Grand Ball Room Banquet 8:00 pm – 10:00 pm — Grand Ball Room

Wednesday

7:00	Registration-Marble Area
7:00 – 8:00	Membership Committee Meeting — Board Room Laboratory and Biosafety Committee Meeting — Soulard
8:00- 10:00	Transformation for Gene Discovery Plant Symposium — Mississippi Tissue Engineering Vertebrate Symposium — Jefferson F
10:00	Coffee Break - Foyer
10:30- 12:30	Gene Transfer Vertebrate Symposium — Jefferson F Tropical Plant Transformation and Tissue Culture Plant Symposium — Mississippi
12:30 – 1:30	2002 Program Committee Meeting — Field
1:00 – 2:30	Disease Resistance Plant Contributed Paper Session — Jefferson A & B
2:45 – 5:00	Tissue Culture and Regeneration Plant Contributed Paper Session — Jefferson A & B
Evening Events	Take Me Out to the Ball Game 5:30 pm — Busch Stadium

Special Events and Meetings

2001 CONGRESS ADDITIONAL MEETINGS AND EVENTS

Saturday Events, June 16	SIVB/ CABI Business Meeting 3:30 pm – 5:00 pm — Board Room IAPTC&B / SIVB Officers Meeting 5:00 pm – 6:00 pm — Jefferson B History Society Meeting 6:00 pm – 7:00 pm — Presidential Suite
Sunday Events, June 17	Education Committee Meeting 12:30 pm – 1:15 pm — Board Room In Vitro – Plant Editorial Board Meeting 12:30 pm – 1:15 pm — Laclede
Monday Events June 18	Plant Section Program Committee Breakfast 7:00 am – 8:00 am — Soulard Strategic Long-range Planning Committee Meeting 7:00 am – 8:00 am — Board Room
Tuesday Events, June 19	Development Committee Meeting 7:00 am – 8:00 am — Board Room Student Affairs Committee Breakfast 7:00 am – 8:00 am — Soulard Lifetime Achievement Reception 12:30 pm – 1:15 pm — Exhibit Hall Poster Breakdown and Removal 2:30 pm – 3:00 pm — Exhibit Hall SIVB Business Meeting 5:00 pm – 6:00 pm — Jefferson B

2001 Congress on In Vitro Biology ~ Schedule of Functions

TIME	TYPE OF FUNCTION	ROOM
FRIDAY, JUNE 15		
5:00 pm – 9:00 pm	SIVB Board of Directors Meeting	Board Room
SATURDAY, JUNE 16		
7:00 am – 7:30 pm	Registration	Marble Area
8:00 am – 12:00 pm	SIVB Board of Directors Meeting	Board Room
8:00 am – 11:00 am	Risk Communication Workshop	Field
8:30 am – 11:00 am	Hands-on Workshop for Teachers – Session I	Clark
11:00 am – 12:00 pm	Student Symposium – In Vitro Research by High School Students	Lewis West
12:00 pm – 4:00 pm	Biotechnology Risk Communication Training	Field
12:00 pm – 4:00 pm	Advanced Tissue Culture Workshop	Lewis East
1:00 pm – 4:00 pm	Hands-on Workshop for Teachers – Session II	Clark
12:00 pm – 1:00 pm	2001 Program Planning Committee Meeting	Laclede
3:00 pm – 6:00 pm	Poster Set-up	Exhibit Hall
3:30 pm- 5:00 pm	SIVB/CABI Business Meeting	Board Room
4:00 pm – 7:00 pm	Monsanto Tour	Monsanto
5:00 pm – 6:00 pm	IAPTC&B / SIVB Officers Meeting	Jefferson B
6:00 pm – 7:00 pm	History Society Meeting	Presidential Suite
7:00 pm – 9:00 pm	Opening Reception (Poster Presentations 7:30 pm – 8:30pm)	Exhibit Hall
SUNDAY, JUNE 17		
7:00 am – 6:00 pm	Registration	Marble Area
7:00 am – 8:00 am	Publications Committee Meeting	Lewis East
7:00 am – 8:00 am	Cell Culture Standardization Meeting	Lewis West
10:00 am– 10:30 am	Coffee Break	Exhibit Hall
10:00 am– 3:00 pm	**Exhibits and Posters** *	Exhibit Hall
12:30 pm- 1:15 pm	Education Committee Meeting	Board Room
12:30 pm – 1:15 pm	In Vitro – Plant Editorial Board Meeting	Laclede
1:15 pm – 2:15	Interactive Poster Sessions	Exhibit Hall
2:15 pm – 2:45 pm	Even Poster Presentations	Exhibit Hall
5:30 pm	Evening at the Missouri Botanical Gardens – Board Buses	Exhibit Hall
6:00 pm – 9:00 pm	Evening at the Gardens/Reception and Dinner	Missouri Botanical Garden
MONDAY, JUNE 18		
7:00 am – 6:00 pm	Registration	Marble Area
7:00 am – 8:00 am	Strategic Long-Range Planning Committee Meeting	Board Room
7:00 am – 8:00 am	Plant Program Committee Breakfast Meeting	Soulard
10:00 am – 10:30 am	Coffee Break	Exhibit Hall
10:00 am – 3:00 pm	**Exhibits and Posters** *	Exhibit Hall
12:30 pm – 1:15 pm	Special Reception Sponsored by Exhibitors and SIVB	Exhibit Hall
1:15 pm – 2:15 pm	Interactive Poster Sessions	Exhibit Hall
2:15 pm – 2:45 pm	Odd Poster Presentations	Exhibit Hall
6:00 pm – 9:00 pm	Plant Section Business Meeting/Social	Mississippi
7:00 pm – 9:00 pm	Vertebrate/Cellular Toxicology Section Meeting/Social	Laclede
TUESDAY, JUNE 19		
7:00 am – 6:00 pm	Registration	Marble Area
7:00 am - 8:00 am	Development Committee Meeting	Board Room
7:00 am – 8:00 am	Student Affairs Committee Breakfast Meeting	Soulard
10:00 am– 10:30 am	Coffee Break	Exhibit Hall
10:00 am – 2:30 pm	**Exhibits and Posters**	Exhibit Hall
12:30 pm – 1:15 pm	Lifetime Achievement Reception	Exhibit Hall
1:15 pm – 2:15 pm	Interactive Poster Sessions	Exhibit Hall
2:15 pm – 2:30 pm	All Poster Presentations	Exhibit Hall
2:30 pm – 3:00 pm	Poster Breakdown and Removal	Exhibit Hall
5:00 pm – 6:00 pm	SIVB Business Meeting	Jefferson B
6:30 pm – 7:30 pm	Reception / Silent Auction	Grand Ball Room
7:30 pm – 10:30 pm	Banquet Dinner	Grand Ball Room
WEDNESDAY, JUNE 20		
7:00 am – 3:30 pm	Registration	Marble Area
7:00 am – 8:00 am	Membership Committee Meeting	Board Room
7:00 am – 8:00 am	Laboratory and Biosafety Committee Meeting	Soulard
12:30 pm – 1:30 pm	2002 Program Committee Meeting	Field
7:00 pm	Take Me Out to the Ballgame	Busch Stadium

Note Additions and changes to functions will be posted on a bulletin board located in the registration area. Please check the bulletin board daily.

*-Poster Viewing from 8:00 am until 9:00 pm Sunday, 6/17 and Monday, 6/18.

SATURDAY, JUNE 16

7:00 am – 7:00 pm	Registration	Marble Area
8:00 am – 12:00 pm	SIVB BOARD OF DIRECTORS MEETING	Board Room

RISK COMMUNICATION WORKSHOP – SCIENTISTS AND THE PUBLIC: HANDLING CONTROVERSIAL ISSUES

Conveners: Cindy Lynn Richard, Council for Agricultural Science and Technology
Maud Hinchee, ArborGen

8:00 am – 11:00 pm	General Workshop	Field

This 3 hour seminar will teach the communication techniques used to discuss controversial scientific issues with the public, with a special emphasis on biotechnology. Attendees will learn about the scientific data that are the basis for risk communication techniques, and how to apply risk communication techniques successfully in their own public speaking situations. This workshop will help scientists gain confidence and greater influence when discussing scientific issues that the public perceives as high risk. Attendees will learn how to apply risk communication techniques when preparing for public presentations, question and answer forums, and interviews with the media.

BIOTECHNOLOGY RISK COMMUNICATION TRAINING – IN-DEPTH TRAINING WHICH MEETS YOUR NEEDS

12:00 pm – 4:00 pm	General Workshop	Field

This 4 hour training session will allow scientists who have attended the morning seminar to practice the techniques of risk communication hands-on in small group settings. Attendees will be asked to identify real-life situations of importance to themselves, and then will be able to practice their communication techniques in mock situations. Each trainee will receive feedback from the training instructors and their work groups; and will have the opportunity to view themselves in action on video recording. Attendees will be able to participate in multiple practice sessions in order to gain as much practical experience and trainer feedback as is possible.

ADVANCED TISSUE CULTURE WORKSHOP: ASSAYS FOR CELL VIABILITY

Convener: Janis Demetrulias, MS Technikos Research Associates

12:00 pm – 4:00 pm	Joint Vertebrate/Invertebrate/Toxicology Workshop	Lewis East

Cell viability is critical in design and data interpretation in cell culture research. However, choosing the most relevant measure for viability is allusive and interpretation of the experimental data is often confounded. Cell viability is defined by the parameter chosen as its measure. Measures range from cellular respiration to cell membrane integrity and the use of specific probes. This half-day session will begin with an overview of cell biology emphasizing pathways critical in cell viability measurement. We will review currently available assays and their relevance and provide examples of the appropriate use of these assays. Ample opportunity will be provided for questions and answers directed to the speakers and for open discussion. Participants will receive copies of all referenced literature.

Saturday, June 16

EDUCATIONAL OUTREACH PROGRAM – A HANDS-ON WORKSHOP TO HELP STUDENTS MEET NEW NATIONAL SCIENCE EDUCATION STANDARDS

SPONSOR: COLGATE-PALMOLIVE COMPANY

Conveners: Zuzana Zachar, State University of New York – Stony Brook
Jennifer Visconti, Northport High School
Patricia Bossert, Northport High School Research Program

8:00 am – 4:00 pm Education Workshop

Classroom-Ready Inquiry Based Modules in Molecular Biology and Plant Tissue Culture
CEU Credit applied for.

8:00 am – 11:00 am **BRINGING DNA TO THE CLASSROOM** Clark

Techniques for DNA isolation and analysis suitable for the secondary classroom will be presented in hands-on workshops. Lab kits and manuals will be provided. High school students who have done these techniques in a secondary classroom setting will be present to discuss their experience and projects.

11:00 am – 12:00pm **STUDENT SYMPOSIUM – IN VITRO RESEARCH BY HIGH SCHOOL STUDENTS** Lewis West

1:00 pm – 4:00 pm **PLANT TISSUE CULTURE IN THE CLASSROOM** Clark

Using a microwave oven or a pressure cooker, supplies found in your kitchen, plus the contents of the newly developed "Kitchen Culture Kit™, Drs. Carol Stiff, Michael Kane, and Ken Torres, will teach you how to mass propagate hundreds of your favorite plants in your kitchen or classroom. This basic technique is used by the nursery industry to mass-produce valueable plant species, and by plant genetic engineers to introduce new genes into plants. A KCK kit with manual and supplies will be provided.

2001 CONGRESS OPENING RECEPTION

7:00 pm – 9:00 pm Exhibit Hall

Saturday, June 16
All Poster Authors will be present
7:30 pm – 8:30 pm
(See list of posters on pages 32-A to 48-A)

SUNDAY, JUNE 17

7:00 am – 6:00 pm Registration Marble Area

ARTIFICIAL CHROMOSOMES

SPONSOR: MONSANTO COMPANY, SYNGENTA

Conveners: Gurdip Brar, Monsanto Agracetus Campus
Alda Vidrich, University of Virginia Health Systems

8:00 am – 10:00 am Joint Vertebrate/Plant Symposium Jefferson F
(See abstracts page 2-A)

The demands of the genome mapping project have led to the development of artificial chromosomes. Yeast Artificial Chromosomes (YACs) first became available in 1984, while bacterial Artificial Chromosomes (BACs) containing human DNA was first reported in 1992, and 1995 saw the appearance of BACs for plant DNA sequences. Their use enabled gene mapping of eukaryotic organisms. The more recent advent of human artificial chromosomes (HACs) has led to studies focusing on gene function and novel approaches to gene therapy. When will plant artificial chromosomes become available? Three of the four building blocks, telomeres, origin of replication, and genes of interest plus marker genes for constructing PAC, are already available. Putative centromere sequences have been identified for Arabidopsis and cloned for potato, rice, and other cereals. However, these sequences await transformation into plants to confirm their function as centromeres. Artificial chromosome technology is rapidly evolving as is the application of this technology. In this SID, leading researchers in the field of plant, yeast, and human artificial chromosomes will provide the current status, projected developments, and future prospectus of this technology.

8:00 Introduction (A.Vidrich)
8:05 J-1 Centromere Structure and Function from Yeast to Arabidopsis
Daphne Preuss, *University of Chicago*
8:35 J-2 Structural and Functional Analysis of a Maize Centomere
James A. Birchler, *University of Missouri-Columbia*
9:05 J-3 Engineering Large Mammalian Artificial Episomal Chromosomes
Jonathan A. Black, *University of North Carolina at Chapel Hill*
9:35 J-4 Development and Application of Artificial Chromosome Expression Systems (ACes)
Edward Perkins, *Chromos Molecular Systems, Inc.*

DEVELOPMENT AND APPLICATION OF CELL MODELS FOR PRE-CLINICAL RESEARCH

Moderator: John Harbell, Institute for In Vitro Sciences

8:00 am – 10:00 am Joint Vertebrate/Toxicology Contributed Paper Session Jefferson D
(See abstracts pages 29-A to 30–A)

8:00 VT-1000 The Effects of Different Plant Protein Hydrolysates on Sp2/0 Cells Expressing Recombinant Pro-urokinase
M. C. Borys, *Abbott Laboratories, K. D. Hughes, and J. M. Ryan*
8:15 VT-1001 A Method for the Synthesis of Stromal Extracellular Matrix (ECM) Synthesized by Normal Human Prostate Cells in Culture
Elizabeth Scotto-Lavino, *State University of New York at Stony Brook, H. L. Sawka, S. R. Simon, and E. J. Roemer*

8:30	VT-1002	Conditional Immortalization of Human Prostate Epithelial and Mesenchymal Cells ***John R. Masters,*** *University College London, M. J. O'Hare, B. Daly-Burns, and D. L. Hudson*
8:45	VT-1003	Population Dynamics of Spheroid Self-Assembly of Prostate Cancer Cells ***Kim C. O'Connor,*** *Tulane University, R. M. Enmon, D. J. Lacks, D. K. Schwartz and R. S. Dotson*
9:00	VT-1004	Activin A Promotes Differentiation of the Salivary Gland Stem Cells into the Acinar Cells ***Miho Furue,*** *Kanagawa Dental College, Y. Zhang, T. Okamoto, R.-I. Hata, and M. Asashima*
9:15	VT-1005	Autonomous and Human Papillomavirus Enhanced Replication of Adeno-Associated Virus Type 2 in an In Vitro Organotypic Culture System ***Craig Meyers,*** *Milton Hershey Medical Center, S. Alam, P. L. Hermonat, and M. Mane*
9:30	VT-1006	13-*cis*-Retinoic Acid Up-Regulates Surface Expression of CD40 on Human Dendritic Cells During Their Differentiation *In Vitro* ***Maurizio Chiriva Internati,*** *Albany Medical College, F. Grizzi, C. Carter, P. Hermonat, and N. Dioguardi*

10:00 am – 10:30 am	Coffee Break	Exhibit Hall
10:00 am – 3:00 pm	Exhibits and Posters	Exhibit Hall

THE DEPLOYMENT OF TRANSGENIC TREES

SPONSOR: ARBORGEN, LLC

Convener: Dave Ellis, Cellfor Incorporated

10:30 am – 12:30 pm	Plant Symposium (See abstracts page 7-A)	Jefferson D & E

With the demand for wood products projected to exceed supply within the next 10 years, interest in tree farming is on the rise. With this interest, comes the realization that genetic engineering can have a significant impact on these tree farms, just as it has with agricultural crops. However, with the use of transgenic trees comes the question of whether long-lived perennial crops have the same or even similar environmental risks as transgenic agricultural crops. In this symposium we explore some of the tissues which may be unique to transgenic trees and begin to put a framework around the regulatory and experimental results collected to date that answer some of the questions posed with the deployment of transgenic trees.

10:30		Introduction (D. Ellis)
10:45	P-1	Assessing the Persistence of DNA from Leaves of Genetically Modified Poplar Trees ***Armand Seguin,*** *Canadian Forest Service*
11:15	P-2	Transgene Dispersal and Control of Flowering in Poplars ***Steven H. Strauss,*** *Oregon State University*
11:45	P-3	Safety Evaluation of Genetically Modified Forestry Products for Global Regulatory Approvals ***Patricia R. Sanders,*** *Colliant, Inc.*

Sunday, June 17

BEYOND THE GENOME: FUNCTIONAL GENOMICS
SPONSOR: AMERSHAM PHARMACIA BIOTECH, AVON PRODUCTS, INTERNATIONAL FOUNDATION FOR ETHICAL RESEARCH, PERKINELMER LIFE SCIENCE

Conveners: Amy Wang, Aventis CropScience
Eugene Elmore, University of California, Irvine

10:30 am – 12:30 pm Joint Vertebrate/Invertebrate/Toxicology Symposium Lewis
(See abstracts pages 2-A to 3-A)

Functional genomics uses genetic sequence information to study the genetic and physiological control of functional pathways of organisms. In the 1980s, functional genomics was only a "futuristic" concept. However, genomic application and techniques are now accepted as an integral part of science. This is due, in part, to the introduction of technological advances in the sequence of various genomes. Among the eukaryotes, sequences for Drosophila, yeast, and more than 20 prokaryotic genomes, were completed in 2000. The expected date for completion of the entire human genome is 2003. The inventory of genes and genome data will impact molecular medicine and improve diagnosis of disease. Prokaryotic genomics will support vaccine design and exploration of new microbial energy sources. Knowledge of other animal and plant genomes offers a new means of pest control, as well as enhancement of crop yield and quality. DNA microarrays are one of the revolutionary technologies employed to study functional genomics. This technology permits the simultaneous screening of the expression of thousands of genes. This process, until recently, was performed with one or two genes at a time. Microarrays are a miniaturized and massive parallel variation of the common Southern, Northern, or Western blots. This symposium will present the methodologies of microarrays and bioinformatics, utilized in functional genomics, and address practical questions of concern to both the novice and experienced researchers.

10:30 Introduction (E. Elmore and A. Wang)
10:45 J-5 Gene Discovery in Plants by Activation Tagging
Helena Mathews, *Exelixis Plant Sciences*
11:10 J-6 Gene Expression Profiles Reveal Effector Pathways of Toxicants
Hisham K. Hamadeh, *National Institute of Environmental Health Science*
11:35 J-7 Proteomics: The View from a 2D Electrophoresis Service Lab
Nancy C. Kendrick, *Kendrick Labs, Inc.*
12:00 J-8 Application of cDNA Microarray to Minute Amount of Biological Samples
Kwong-Kwok Wong, *Pacific Northwest National Laboratory*

THE PROTEOMICS APPROACH TO UNDERSTANDING A BIOLOGICAL SYSTEM
SPONSOR: AMERSHAM PHARMACIA BIOTECH

Convener: G. Reid Ashbury, Amersham Pharamcia Biotech

12:30 pm – 1:30 pm Joint Vertebrate/Invertebrate/Toxicology Workshop Clark

A combination of protein structure, function and protein-protein interactions, proteomics, is critical to understanding the key intracellular and intercellular mechanisms involved in biological systems. These fundamental building blocks of knowledge will enable researchers to target disease states and drug discovery from a new and more informed point of view. Given the complexity of the proteome compared to the genome an overwhelming number of samples and resulting data will be generated from these studies. To cope with this high throughput, new technologies and methods have been developed. Using 2D electrophoresis, spot handling equipment and mass spectrometry proteins from complex mixtures are identified and characterized.

Sunday, June 17

SECONDARY METABOLISM

Moderator: Mary Ann Lila Smith, University of Illinois

1:15 pm – 2:15 pm — Joint Interactive Plant/Toxicology Poster Session (See list of posters on pages 31-A to 32-A) — Exhibit Hall

DISEASE RESISTANCE

Moderator: C. S. Prakash, Tuskegee University

1:15 pm – 2:15 pm — Interactive Plant Poster Session (See list of posters on page 34-A) — Exhibit Hall

IN VITRO TOXICOLOGY

Moderator: Eugene L. Elmore, University of California – Irvine

1:15 pm – 2:15 pm — Joint Interactive Vertebrate/Toxicology Poster Session (See list of posters on pages 44-A to 45-A) — Exhibit Hall

Sunday, June 17
Even Poster Authors will be present
2:15 pm – 2:45 pm
(See list of posters on pages 32-A to 48-A)

PLENARY SESSION

CO-SPONSORED BY DUPONT AGRICULTURAL PRODUCTS, MONSANTO COMPANY

Convener: Todd Jones, DuPont AgBiotech

3:00 pm – 4:30 pm — Plenary Session — Missouri/Illinois

Opportunities and Challenges in Plant Biotechnology to Benefit Health and Sustainability
(See abstract page 1-A)

3:00 pm — Introduction: Todd Jones, 2001 Congress Program Chair
Opening Remarks: Mary Ann Lila Smith, University of Illinois, and President, Society for In Vitro Biology

PS-1 — Plenary Speaker: ***Roger N. Beachy,*** *President of the Donald Danforth Plant Science Center and Co-director of the International Laboratory for Tropical Agricultural Biotechnology*

6:00 pm – 9:00 pm — Plenary Reception and Dinner — Missouri Botanical Gardens

CO-SPONSORED BY DUPONT AGRICULTURAL PRODUCTS, MONSANTO COMPANY

MONDAY, JUNE 18

7:00 am – 6:00 pm Registration Marble Area

INSECT HORMONES AND APPLICATIONS FOR PEST CONTROL

SPONSOR: AVENTIS CROPSCIENCE, SYNGENTA CROP PROTECTION AG, ROHM AND HAAS COMPANY

Convener: Guy Smagghe, Free University of Brussels-Brussels, Ghent University

8:00 am – 12:30 pm Invertebrate Symposia Field

(See abstracts pages 5-A to 6–A)

Growth and development in insects is achieved by periodic shedding of the old exoskeleton. This molting process involves production of new structures, remodeling of larval tissues and death of others, and the coordination is under the control of the insect hormones 20-hydroxyecdysone (20E) and juvenile hormones (JHs). The molecular target for 20E consists of a heterodimer of two proteins, the ecdysone receptor (ECR) and the product of another gene, ultraspiracle (USP), an insect homolog of vertebrate retinoid X receptor. The receptor for JHs remains elusive so far. As a way of insect pest control, any interference in the homeostasis of one or more hormones or in the various hormone-dependent processes with analogs (agonists or antagonists) would result in disruption or abnormal growth and development of the target pest insect. As Caroll Williams already suggested in 1967, compounds that mimic the action of insect hormones can be used as safe insecticides and help to overcome insecticide resistance. This indeed has been the basis for the development by the agrochemical industry of new, target pest selective insecticides with a JH and ecdysone mode of action. After an introductory survey on the physiological and molecular activities of 20E and JHs, attention is given to the importance of in vitro biology and biotechnology as tools for studying hormone action and subsequently in the selection of novel insecticide candidates. This session will also concentrate on pharmacological and pharmacokinetic modeling and screening for new insecticide hormone analogs.

8:00 Introduction (G. Smagghe)

8:15 I-1 Hormonal Regulation of the Transcriptional Cascade Leading to Dopa Decarboxylase Expression
***Kiyoshi Hiruma,** University of Washington*

8:45 I-2 Non-steroidal Ecdysone Agonists: In Vitro Methods for Discovery and Use for Agriculture and Pharmaceutical Markets
***Tarlochan S. Dhadialla,** Rohm and Haas Co.*

9:15 I-3 Mode of Action, Specificity, and Possible Resistance Mechanism of Non-steroidal Ecdyson Analogs
***Arthur Retnakaran,** Canadian Forest Service*

9:45 Discussion

10:00 am – 10:30 am Invertebrate Session Coffee Break Exhibit Hall

10:30 I-4 In Vitro Imaginal Disc Cultures as Bioassay for Ecdysone Action Field
***Guy Smagghe,** Ghent University-Ghent*

11:00 I-5 Comparative Structure-Activity Relationship of Various Non-steroidal Ecdysone Agonists Between In Vivo and In Vitro Assay Systems
***Yoshiaki Nakagawa,** Kyoto University*

11:30 I-6 Development of New Screening Systems for Hormonal Compounds Using Transformed Insect Cell Lines.
***Luc Swevers,** National Centre for Scientific Research Demokritos*

12:00 I-7 Current and Future Use of Insect Growth Regulators in Crop Protection
***Hartmut Kayser,** Syngenta Crop Protection AG*

REPRODUCTIVE HEALTH: ASSESSING THE ROLE OF ENDOCRINE DISRUPTORS IN VITRO

Conveners: James W. DuMond, Jr., University of Alabama at Birmingham School of Public Health
Deodutta Roy, University of Alabama at Birmingham

8:00 am – 10:00 am | Toxicology Symposium | Laclede

(See abstracts page 14–A)

Over the last two decades, researchers have revealed an overwhelming number of chemicals that can disrupt the endocrine system. Estrogen pathways have been a primary research focus during this period, however, perturbation of other hormone cascades has been reported. Since a disruption of the endocrine system can be both detrimental and beneficial to the host, industry has been increasingly burdened with the task of testing their products for endocrine disrupting properties as well as screening chemicals for drug development. Thus the focus of this session will be a review of the traditional and novel *in vitro* techniques used in assaying chemicals for estrogenic properties as well as their effect on reproductive health.

8:00 Introduction (D. Roy)

8:15 T-1 A Reduction of DNA Repair Capacity by Endocrine Disruptors in Testicular Cells
James W. Dumond, Jr., *University of Alabama at Birmingham*

8:40 T-2 In Vitro Assessment of Endocrine Disruptors: Activity of the Environmental Estrogen Bisphenol A at Levels of Current Human Exposure
Wade V. Welshons, *University of Missouri-Columbia*

9:05 T-3 Use of an In Situ Ovarian Cell System to Study Effects of Phyto- and Synthetic Estrogens on Apoptosis
Todd A. Winters, *Southern Illinois University*

9:30 T-4 Detection of Environmental and Occupational Estrogenic Chemicals–Induced Mutations in Mouse Leydig Cells by RAPD/AP-PCR Fingerprinting
Kamaleshwar P. Singh, *University of Alabama at Birmingham*

APPLICATIONS OF IN VITRO CULTURE FOR HABITAT RESTORATION

SPONSOR: DOW AGROSCIENCES, LLC

Convener: Michael Kane, University of Florida

8:00 am – 10:00 am | Plant Symposium | Jefferson B & C

(See abstracts pages 7-A to 8-A)

Numerous federal and state statutes require restoration of ecological function of degraded wetlands and mined lands, or replacement of destroyed wetlands (mitigation). This is typically accomplished through extensive planting and successful establishment of herbaceous and woody species. Currently, there are many challenges to successful habitat restoration/creation. These include maintenance of genetic diversity, plant source problems, low survival of poorly adapted ecotypes, and attainment of ecological structure and function. During this symposium, applications of in vitro plant technology for selection of naturally and induced genetic variability, enhanced stress tolerance, propagation, and storage of plants used for habitat restoration and creation will be presented.

8:00 Introduction (M. Kane)

8:15 P-4 In Vitro Culture for Habitat Revegetation: Issues & Opportunities
Michael E. Kane, *University of Florida*

8:40 P-5 Exploring Natural and Tissue Culture-induced Plant Genetic Diversity for Salt Marsh Creation
Denise M. Seliskar, *University of Delaware*

9:05 P-6 Tissue Culture and Wetland Establishment of the Freshwater Monocots *Carex, Juncus, Scirpus,* and *Typha*
Suzanne M. D. Rogers, *Salem International University*

9:30 P-7 Biotechnological Approaches to Habitat Revegetation: A Commercial Perspective
Brent Zettl, *Prairie Plant Systems, Inc.*

DICOT TRANSFORMATION

Moderator: Kim Rayford, Monsanto Company
S. Jayasankar, University of Florida

8:00 am – 10:00 am — Plant Contributed Paper Session — Mississippi
(See abstracts pages 22-A to 23-A)

8:00 P-1000 Expression of a GFP Fusion Marker Under the Control of Three Constitutive Promoters and Enhanced Derivatives in Transgenic Grape
S. Jayasankar, *University of Florida, Z. Li, and D. J. Gray*

8:15 P-1001 High Efficiency Transformation of Egg Plant (*Solanum melongena* L.) by Agrobacterium tumefaciens
Gregory Franklin, *Indian Institute of Science, and G. Lakshmi Sita*

8:30 P-1002 MicroTom – A Model Functional Genomics Assay
Yinghui Dan, *Monsanto Company, H. Yan, T. Munyikwa, J. Dong, B. Zhang, L. K. Lahman, and C. Rommens*

8:45 P-1003 Tomato Fruit with Enhanced Calcium Nutrition
Sung Hun Park, *Texas A&M University, K. D. Hirschi, J. E. Park, and R. H. Smith*

9:00 P-1004 Transformation of Multiple Genes into Soybean (*Glycine max* (L.) Merrill) by Co-bombardment and by a 6-Gene Cluster Plasmid
Monica A. Schmidt, *University of Georgia, B. J. Artelt, and W. A. Parrot*

9:15 P-1005 GFP Introduction, Expression, and Possible Toxicity in Soybean
John J. Finer, *The Ohio State University, K. M. Larkin, and M. Buenrostro-Nava*

10:00 am – 10:30 am — Coffee Break — Exhibit Hall

10:00 am – 3:00 pm — Exhibits and Posters — Exhibit Hall

NUTRACEUTICALS/EDIBLE VACCINES

SPONSOR: MONSANTO COMPANY, PROTEIN TECHNOLOGIES INTERNATIONAL

Conveners: Schuyler Korban, University of Illinois
Marceline Egnin, Tuskegee University

10:30 am – 12:30 pm — Plant Symposium — Jefferson B & C
(See abstracts pages 8-A to 9-A)

Using the tools of biotechnology, plants are being targeted for genetic manipulation and/or enhancement for the production, synthesis, and delivery of biopharmaceuticals and nutraceuticals which will significantly contribute to protection against disease and/or improvement of both human and animal health and their overall well-being. Protecting human and animal populations against pathogenic agents by developing vaccines that are produced in plants will have significant impact on the field of plant biotechnology and biological 'pharming'. Harnessing the potential of plants for production of nutraceuticals will also greatly enhance the new important role of plants as functional foods.

This session will focus on advances in developing plant-based vaccines against human and mammalian pathogens and their role as well as those of biologically-active (nutraceutical) compounds in improving mammalian health and the under-
lying immune systems.

10:30		Introduction (S. Korban and M. Egnin)
10:45	P-8	Plant-based Vaccines: Expression and Oral Immunogenicity ***Hugh S. Mason,*** *Boyce Thompson Institute for Plant Research at Cornell University*
11:15	P-9	Plant Viruses as an Alternative System for Expression of Foreign Sequences ***Vidadi Yusibov,*** *Thomas Jefferson University*
11:45	P-10	Conjugated Linoleic Acid: A Nutraceutical with Immunomodulatory Properties ***Josep Bassaganya-Riera,*** *Iowa State University*

ABC TRANSPORTERS: THEIR ROLE IN MULTI-DRUG RESISTANCE, BIOAVAILABILITY, AND DRUG-DRUG INTERACTIONS

SPONSOR: ELI LILLY AND COMPANY

Convener: Dennis Laska, Eli Lilly and Company

10:30 am – 12:30 pm — Toxicology Symposia — Laclede

(See abstracts page 15-A)

An area of growing interest in cellular biology as well as the pharmaceutical industry is the functional investigation of ATP dependent, transmembrane transporter protein series called the ABC super family. This ABC super family includes the multi-drug resistance protein series (mdr's), the multi-resistance protein series (MRP's), as well as anionic and cationic transporters located on the apical and/or basolateral membranes of highly polarized epithelial and endothelial cells. They may play a role in cellular homeostasis which makes their expression in many tissues appears to be ubiquitous. However, they are also subject to up-regulation and over expression in multi-drug resistant malignacies and sites of drug uptake and excretion (e.g., liver, kidney, intestine). The cellular role of trafficking xenobiobics provides a platform for study of drug resistant tumors, limited oral bioavailability of pharmaceutics, penetration across the blood-brain barrier, and drug-drug interactions and accumulation in tissues such as liver and kidney. This symposium will provide a broad overview with specific examples of the contemporary, functional understanding of these important transmembrane proteins.

10:30		Introduction (D. Laska)
10:45	T-5	Transporter Localization and Drug Disposition in Multi-drug Resistant Cancer Cells ***Daniel C. Williams,*** *Eli Lilly and Company*
11:15	T-6	The MRP Subfamily of Drug Transporters ***Gary D. Kruh,*** *Fox Chase Cancer Center*
11:45	T-7	Drug Uptake and Efflux Transporters: *In Vitro* to I*n* V*ivo* Relevance ***Richard B. Kim,*** *Vanderbilt University School of Medicine*

12:30 pm – 1:15 pm — **Reception in Exhibit Hall** — **Exhibit Hall**

Exhibitors/SIVB Reception

Monday, June 18

DICOT TRANSFORMATION

Moderator: John J. Finer, The Ohio State University

1:15 pm – 2:15 pm — Interactive Plant Poster Session — Exhibit Hall
(See list of posters on pages 35A to 36-A)

PART I: EVALUATION OF CRYOPRESERVATION TECHNIQUES
PART II: HUMAN EPIDERMAL KERATINOCYTES

Moderator: William J. Smith, US Army Medical Research Institute of Chemical Defense

1:15 pm – 2:15 pm — Joint Interactive Vertebrate/Toxicology Poster Session — Exhibit Hall
(See list of posters on pages 45-A to 46-A)

Monday, June 18
Odd Poster Authors will be present
2:15 pm – 2:45 pm
(See list of posters on pages 32-A to 48-A)

REGULATORY AFFAIRS/PUBLIC ACCEPTANCE

SPONSOR: MONSANTO COMPANY, UST, INC.

Conveners: Laura Privalle, Syngenta Seeds, Inc.
Maud Hinchee, ArborGen

3:15 pm – 5:45 pm — Plant Symposium — Jefferson B & C
(See abstracts pages 9-A to 10-A)

Biotechnology has had a tremendous impact on man's ability to develop crop plants with new valuable traits. In the United States, approximately 60% of the soybean acres and 30% of the corn acres are planted in varieties which contain biotech traits which benefit the economics of agricultural production. Many new technologies that impact the food supply, when introduced widely, trigger a public reaction concerning the risks associated with the new technology. The public requires assurance that such a technology provides benefits to society and the environment which outweigh any perceived risks. Governments provide regulatory oversight of technologies and governmental regulatory processes evolve with the implementation of these technologies. Two speakers in this session will address the regulatory system which is in place in the United States, and globally, to limit risks with the introduction of biotech crops. Two speakers will address the public reaction to plant biotech products and the necessary public information requirements to address the public reaction. All speakers will address how the scientific community should participate in the public understanding of the benefits and risks of plant biotechnology.

3:15 — Introduction (M. Hinchee and L. Privalle)

3:30 P-11 — Scientific, Regulatory, and Communication Issues in Global Perspective
Robin Woo, *Georgetown Center for Food and Nutrition Policy*

3:55 P-12 — Federal Coordinated Framework for the Regulation of Biotechnology in the United States
David S. Heron, *USDA*

4:20 P-13 — "Scientist Communicator" Shouldn't be an Oxymoron: Understanding Our Role in the Food and Agricultural Biotechnology Dialog
Cindy Lynn Richard, *Council for Agricultural Science and Technology*

4:45 P-14 The National Research Council Committee on Agricultural Biotechnology, Health, and the Environment
Barbara Schaal, *Washington University*

5:10 P-15 Consumer Perspectives on Food Biotechnology
Cheryl Toner, MS, RD, *International Food Information Council*

THE IMPORTANCE OF CONTROLS FOR IN VITRO BIOLOGY

Convener: John Harbell, Institute for In Vitro Sciences, Inc.

3:30 pm – 5:30 pm Joint Vertebrate/Toxicology Workshop Laclede
(See abstracts page 19–A)

One hallmark of cell culture-based studies is the ability, in fact the requirement, to develop an experimental design that can specifically control a specific variable in a specific manner. The selection of proper controls and the interpretation of the observed differences between experimental groups are often difficult. Some of the reasons include complex interactions within the culture system, between the test system and the test materials, and between the individual kinetic and transport processes which all determine the relevance of the in vitro system to the tissue or organ system in vivo. This workshop will examine controls in the broadest sense. First, the selection of controls to facilitate understanding of the complex interactions between physical and biochemical parameters in the bioreactor and the guidance such systems provide to smaller scale culture. Second, assay controls in the classic sense which help assure relevance of the end points measured and the consistency in the system. Third, macro controls or prediction models that show the overall relevance of the in vitro system to the prediction of the in vivo mode of action.

3:30 Introduction (J. Harbell)

3:40 W-1 The Challenge of Choosing Controls for Bioreactor Studies of Cells and Tissues
Gordana V. Vunjak-Novakovic, *Massachusetts Institute of Technology*

4:15 W-2 The Importance and Application of the Prediction Model to In Vitro Biology
Leon H. Bruner, *Gillette Medical Research Laboratories*

4:50 W-3 The Need for Controls Focused on an Assay's End Points
John W. Harbell, *Institute for In Vitro Sciences*

TUESDAY, JUNE 19

7:00 am – 6:00 pm Registration Marble Area

NOVEL BREEDING STRATEGIES

SPONSOR: RICETEC, INC., UST, INC.

Conveners: Melissa Heatley, Rice Tec Inc.
Peggy Ozias-Akins, University of Georgia

8:00 am – 10:00 am Plant Symposium Missouri
(See abstracts pages 10-A to 11-A)

The unique ability of some plants to develop without fertilization has been investigated for the past century. Gametophytes that do not participate in fertilization can give rise to a new sporophytic generation that either contains the entire maternal chromosome complement (apomixis) or only half the maternal (gynogenesis) or paternal (androgenesis) chromosome set. Traditional plant breeders have, over the years, attempted to exploit these respective characteristics for the purpose of fixing heterosis or more rapidly obtaining inbreds. Only in the more recent past have scientists made the production of doubled haploids more feasible, resulting in practical application of techniques like anther and ovary culture to breeding schemes. Although apomixis has not been as readily applicable to a wide range of species, its potential for fixing hybrid vigor has generated considerable interest in understanding the genetic mechanisms underlying the trait. Each speaker in this symposium is exploring these novel strategies for current and future application to breeding programs.

8:00 Introduction (M. Heatley and P. Ozias-Akins)
8:15 P-16 Using Apomixis in Crop Breeding and Genetics
Wayne W. Hanna, *USDA-ARS Crop Genetics and Breeding*
8:45 P-17 Haploid Methods in Wheat and Their Application in Western Canada
Julian B. Thomas, *Agriculture and AgriFood Canada*
9:15 P-18 Towards the Induction of Apomixis: Manipulating Sexual Reproduction in Flowering Plants
Jean-Phillipe Vielle-Calzada, *CINVESTAV*

UNDERSTANDING THE BASIS OF STEM CELL PLURIPOTENCY/PLANT CELL TOTIPOTENCY

SPONSOR: LIFE TECHNOLOGIES – A DIVISION OF INVITROGEN, DEFENSE ADVANCED RESEARCH PROJECTS (DARPA), UST, INC.

Conveners: Mindy Fitter, Monsanto
Paul Price, Life Technologies, Inc.
Patrick R. Hughes, Boyce Thompson Institute

8:00 am – 10:00 am Joint Vertebrate/Invertebrate/Plant Symposium Jefferson B & C
(See abstracts pages 3-A to 4-A)

During embryogenesis a single cell gives rise to a multicellular organism whose cells and tissues have diverse characteristics and function. A cell that can form an entire organism is referred to as "totipotent". More restricted cells that reside within a tissue or organ but are still able to choose from multiple pathways of differentiation are called "pluripotent stem cells". Stem cells can be maintained and propagated in vitro and guided to form various cell types and tissues. Today's research is trying to understand the environment, external signals, and intrinsic factors that regulate the pathways resulting in differentiation. New discoveries are pointing to the roles of negative and positive transcription factors and environmental signals in cellular differentiation that cross the different classes of living organisms. This

symposium will bring together speakers utilizing plant, vertebrate, and invertebrate cells in order to explore what sends a cell down a specific pathway of differentiation.

8:00 Introduction (M. Fitter, P. Price, and P. R. Hughes)
8:15 J-9 From How Many Different Cells Can a Plant Make an Embryo?
Kim Boutilier, *Plant Research International*
8:45 J-10 Molecular Control of Muscle and Heart Development During Drosophila Embryogenesis
Alan Michelson, *Brigham and Women's Hospital*
9:15 J-11 Characterization and Differentiation of Human Embryonic Stem Cells
Melissa Carpenter, *Geron Corporation*

FRONTIERS IN LOW-TEMPERATURE PRESERVATION OF CELLS AND TISSUES

SPONSOR: ORGAN RECOVERY SYSTEMS, INC., SOCIETY OF CRYOBIOLOGY, THERMOFORMA SCIENTIFIC

Conveners: Lia Campbell, Organ Recovery Systems, Inc.
Michael Taylor, Organ Recovery Systems, Inc.

8:00 am – 10:00 am Joint Vertebrate/Toxicology Workshop Laclede
(See abstracts pages 19A to 20-A)

The field of Cryobiology is often misunderstood and oversimplified. A number of factors can affect the health of cells and tissues stored below physiological temperature and must be considered for successful storage and to obtain normal, healthy cells and tissues after storage. This session provides those who are unfamiliar with cryobiology a basic understanding of low temperature storage of cells and tissues. Topics to be reviewed include an overview of the fundamentals of cryopreservation, vitrification, the perils of ice formation and ways to avoid it as well as how low-temperature storage affects the overall health of the cell. Finally, with tissue-engineering becoming more and more commonplace, cryobiology will be discussed in relation to the long-term storage of engineered tissues.

8:00 Introduction(M.Taylor)
8:15 W-4 Fundamentals of Classical Cryopreservation
Locksley E. McGann, *University of Alberta*
8:40 W-5 Apoptoptic Proteolytic Cascades to Cryopreservation-induced Delayed-onset Cell Death
John M. Baust, *University of Binghamton*
9:05 W-6 Cryopreservation with the Avoidance of Ice
Michael J. Taylor, *Organ Recovery Systems, Inc.*
9:30 W-7 Use of Intracellular Sugars for Stabilization of Mammalian Cells in Dried State
Mehmet Toner, *Shriner's Research Institute*

10:00 am – 10:30 am Coffee Break Exhibit Hall

10:00 am – 3:00 pm Exhibits and Posters Exhibit Hall

PRIMARY HUMAN CELL CULTURES OR IMMORTALIZED CELLS: MODELS FOR USE IN HUMAN TOXICOLOGY AND DISEASE STUDIES

Convener: William J. Smith, USAMRICD

10:30 am – 12:30 pm Joint Vertebrate/Toxicology Workshop Laclede

Human cell cultures and explanted tissues play central roles in the study of human disease processes and pathogenic mechanisms of toxicity. Many laboratories use surgical biopsy material or commercial sources of primary cell cultures as in vitro models. Such material can be costly and lack reproducibility, but are thought to provide the closest in vitro correlates to the in vivo processes of interest. The cell culturist has a substantial inventory of continuous cell lines available from commercial sources and cell banks. In addition, there are batteries of techniques for creating immortalized cell lines that can alleviate some of the cost issues and provide long-term cultures with reproducible biochemical profiles. The roundtable will address questions of availability and utility of cells for use in human studies and will posit those factors that researchers should consider in building their in vitro models to assure reliability of endpoints and extrapolative ability of their data to the human subject.

Speakers: ***John Harbell,*** *Institute for In Vitro Sciences*
Soverin Karmiol, *BioWhittaker, Inc.*
Robert Hay, *American Type Culture Collection*
Shigeru Yasumoto, *Kanagawa Cancer Center*
R. Ian Freshney, *University of Glasgow*

ROOTING OF MICROPROPAGATED PLANTS

SPONSOR: THE SCOTTS COMPAN Y

Conveners: Valerie Pence, Cincinnati Zoo and Botanical Gardens
Yongjian Chang, North American Plants, LLC

10:30 am – 12:30 pm | Plant Symposium | Missouri

(See abstracts page 11-A)

Rooting of in vitro shoots is a critical step in the production of micropropagated plants and one which has often been difficult to achieve. This session will explore rooting in both herbaceous and woody species and will examine the effects of growth regulators, maturation, and other factors which can inhibit the initiation of roots.

10:30 Introduction (V. Pence and Y. Chang)

10:45 P-19 Rooting of Microcuttings: Theory and Practice
Geert-Jan de Klerk, *Centre for Plant Tissue Culture Research*

11:15 P-20 In Vitro Shoots Should Be Easy to Root?
Tim Marks, *Horticulture Research International*

11:45 P-21 Influence of Stage II Cytokinin Selection on Rooting and Acclimatization of Native Coastal and Wetland Plants
Michael E. Kane, *University of Florida*

MONOCOT TRANFORMATION

Moderator: Jeremy Bell, The Noble Foundation
Camri Langbecker, Monsanto Company

10:30 am – 12:30 pm | Plant Contributed Paper Session | Jefferson B & C

(See abstracts pages 23-A to 24-A)

10:30 P-1006 Use of Barley Endosperm-specific Hordein Promoters for Production of Recombinant Proteins in Transgenic Cereal Seeds
Myeong-Je Cho, *University of California – Berkeley, B. B. Buchanan, and P. G. Lemaux*

10:45 P-1007 Herbicide and Insect Resistance in Transgenic Rice
Sung Hun Park, *Texas A&M University, K. D. Hirschi, J. E. Park, and R. H. Smith*

11:00	P-1008	Generation and Evaluation of Transgenic Tall Fescue Plants ***Zengyu Wang,*** *The Noble Foundation, J. Bell, D. Lehmann, M. Scott, C. Auh, P. Dowling, and A. Hopkins*
11:15	P-1009	Plant Regeneration and Genetic Transformation of Russian Wildrye ***Jeremy Bell,*** *The Noble Foundation, D. Lehmann, M. Scott, A. Hopkins, and Z. Wang*
11:30	P-1010	Identification of a Highly Transformable Wheat Genotype for Mass Production of Fertile Transgenic Plants ***Alessandro Pellegrineschi,*** *CIMMYT, L.M. Noguera, S. McLean, B. Skovmand, R. M. Brito, L. Velazquez, R. Hernandez, M. Warburton, and D. Hoisington*
11:45	P-1011	Desiccation of Agrobacterium-inoculated Pre-cultured Plant Tissues Significantly Enhances T-DNA Delivery and Subsequently Increases Stable Transformation in Wheat ***Ming Cheng,*** *Monsanto Company, T. Hu, J. Layton, C.-N. Liu, and J. E. Fry*

MONOCOT TRANSFORMATION

Moderator: Bob V. Conger, University of Tennessee

1:15 pm – 2:15 pm	Interactive Plant Poster Session (See list of posters on pages 36-A to 37-A)	Exhibit Hall

OCULAR MODELS

Moderator: Sim F. Webb, University of East Anglia

1:15 pm – 2:15 pm	Joint Interactive Vertebrate/Toxicology Poster Session (See list of posters on page 47-A)	Exhibit Hall

Tuesday, June 19
All Poster Authors will be present
2:15 pm – 2:30 pm
(See list of posters on pages 32-A to 48-A)

2:30 pm – 3:00 pm	Poster Breakdown and Removal	Exhibit Hall

INDUCIBLE GENE SYSTEMS
SPONSOR: SYNGENTA

Conveners: Nancy Reichert, PhD, Mississippi State University
Allan Wenck, Syngenta

3:00 pm – 5:00 pm | Plant Symposium | Missouri

(See abstracts pages 11-A to 12-A)

Most transgenic plants currently on the market contain strong constitutive promoters, such as CaMV35S. Such continuous expression would be non-desirable if the gene product was only needed at specific times as it wastes energy. An example of such would be disease resistance transgenes that are expressed whether the specific pathogens are present or not. In addition, expression of certain genes in all tissues and at all stages of development may be detrimental to plant health and final yield. Chemical gene induction systems have been identified and characterized in various organisms. An ideal, simplified system would display low/no basal expression and a fast, large-fold induction of activity upon application of the inducer. The inducer would be non-toxic, inexpensive and easy to apply. As such, chemically-induced transgene expression could enable precise regulation and be an extremely useful research tool in many areas of plant biology. This symposium will present an overview of chemically induced gene expression systems and then focus on promising systems currently being analyzed in transgenic plants.

3:00		Introduction (N. Reichert and A. Wenck)
3:15	P-22	Chemically Regulated Zinc Finger Transcription Factors ***Ulrich G. Schopfer,*** *Novartis Pharma AG*
3:40	P-23	The XVE Inducible Expression System and its Applications in Plant Biotechnology ***Jianru Zuo,*** *Rockefeller University*
4:05	P-24	A Chemical Gene Switch in Maize Using the Insect Ecdysone Receptor ***Scott Valentine,*** *Syngenta*
4:30	P-25	The alc Gene Switch: Towards Use in the Field ***Alberto Martinez,*** *Syngenta*

TOXICOLOGICAL APPLICATIONS OF COMMERCIALLY AVAILABLE EPITHELIAL MODELS
SPONSOR: JOHNSON AND JOHNSON

Conveners: Janis Demetrulias, MS Technikos Research Associates
Amy Wright, Ciba Vision

3:00 pm – 5:00 pm | Toxicology Symposium | Laclede

(See abstracts pages 15-A to 16-A)

Many programs in basic and applied research require *in vitro* model systems that retain the normal spatial arrangement and differentiation of the cells *in vivo*. In studies of skin, *ex vivo* organ culture was often employed but tissue availability and reproducibility were severe complications. Tissue engineering has produced successful skin constructs for clinical and research applications. Since the technology required to produce reproducible tissue is complex and often proprietary, it is often most efficient to turn to commercial tissue sources. This session will examine the role of commercially available tissue models in evaluating the impact of materials on surface epithelia. Unifying principles of skin cell biology and their relevance to endpoints used in these evaluations will be discussed in relationship to the various tissue constructs available.

3:00		Introduction (J. Demetrulias and A. Wright)
3:15	T-10	TBA ***Arnold Caplan,*** *Case Western University*
3:45	T-8	Selection and Use of Appropriate Skin and Epithelial Models for Product Testing ***Katherine M. Martin,*** *Johnson & Johnson*

4:15 T-9 Evaluation of the Usefulness of 3-D-models of Reconstituted Human Skin and Epidermis in Applications of Regulatory Skin Toxicology: Prevalidation, Validation, Catch-Up-Validation, and Regulatory Acceptance.
Manfred Liebsch, *ZEBET, Berlin, Germany*

INVERTEBRATE CELLS

Moderator: Cynthia Goodman, USDA ARS BCIRL

3:00 – 4:00 pm — Invertebrate Contributed Paper Session — Jefferson C
(See abstracts page 21-A)

3:00 I-1000 Stem Cells from Insect Midgut Cultures Differentiate in Response to Two New Peptides from Insect Hemolymph
Marcia J. Loeb, *USDA, and H. Jaffe*

3:15 I-1001 Differences in Production Levels of HzSNPV in Low and High Passages of the *Heliothis virescens* Cell Line HvAM1
Cynthia L. Goodman, *USDA ARS BCIRL, A. H. McIntosh, J. J. Grasela, S. G. Saathoff, and C. I. Ignoffo*

3:30 I-1002 Application of DNA Microarray Technology for Gene Discovery and Expression Analysis in a Non-model Organism
Shirley A. Pomponi, *Harbor Branch Oceanographic Institution, R. Willoughby, and C. G. Russell*

5:00 – 6:00 pm — **SIVB Business Meeting** — Jefferson B
(All Members are Urged to Attend)

6:30 – 7:30 pm — **Reception/Silent Auction** — Grand Ballroom

7:30 – 10:30 pm — **Banquet Dinner** — Grand Ballroom
Seating is limited. Admittance to Banquet by Advance Ticket Holders only.

WEDNESDAY, JUNE 20

7:00 am – 3:30 pm | Registration | Marble Area

TISSUE ENGINEERING

SPONSOR: BIOWHITTAKER

Convener: Gordana Vunjak-Novakovic, Massachusetts Institute of Technology

8:00 am – 10:00 am | Vertebrate Symposium | Jefferson F

(See abstracts page 17-A)

Tissue engineering has been motivated by the need to create functional biological substitutes of living tissues that can maintain, improve, or restore tissue function. Ideally, the cells within engineered tissues must exhibit the appropriate pattern of gene expression, and the tissues must develop with the appropriate structure. Two components, in addition to the cells themselves, are essential for this approach to be successful. (1) Synthetic biodegradable polymer scaffolds are required to provide a structural template for tissue formation, and convey chemical and physical regulatory signals to the cells in the engineered tissues. (2) Tissue culture bioreactors are required to provide a controlled physiological environment that embodies the regulatory signals for the development of functional tissue structures. In addition, any strategy of creating a functional substitute of a native tissue must involve the recapitulation of certain aspects of embryonic tissue development. This session will discuss the key requirements for engineering functional tissues, including the coordinated use of cells, polymeric scaffolds, and regulatory signals (genetic, physical, chemical), as well as recent progress in quantitative and modeling studies of the structure and function of engineered tissues, in vitro and in vivo.

8:00		Introduction (G. Vunjak-Novakovic)
8:15	V-1	Tissue Engineered Cell Therapy for Skeletal Tissues ***Arnold I. Caplan,*** *Case Western University*
8:45	V-2	TBA ***Linda Griffith,*** *Massachusetts Institute of Technology*
9:15	V-3	Tissue Engineering Bioreactors ***Gordana V. Vunjak-Novakovic,*** *Massachusetts Institute of Technology*

TRANSFORMATION FOR GENE DISCOVERY

SPONSOR: GARST SEEDS COMPANY, RICETEC, INC.

Conveners: Mark Jordan, Agriculture & Agri-Food Canada
Alessandro Pellegrineschi, CIMMYT

8:00 am – 10:00 am | Plant Symposium | Mississippi

(See abstracts pages 12A to 13A)

Gene transfer technology in certain model species such as Arabidopsis or tobacco has progressed to the point where a very large number of independent transformation events can be rapidly obtained. This has facilitated functional genomics and gene discovery programs through techniques such as T-DNA tagging, activation tagging, and gene silencing. Use of such techniques for other species requires the development of either high throughput transformation systems or novel approaches such as alternative vectors or transposon tagging schemes. This session will cover transformation technology and activation tagging in a model legume, the development of viral vectors for functional genomics, and a new transposon tagging system for cereals.

Wednesday, June 20

8:00		Introduction (M. Jordan and A. Pellegrineschi)
8:15	P-26	In Planta Transformation and Insertional Mutagenesis in *Medicago truncatula* ***Maria J. Harrison,*** *The Samuel Roberts Noble Foundation*
8:45	P-27	Gene Function Discovery with Plant Viral Vectors ***Guy della-Cioppa,*** *Large Scale Biology Corporation*
9:15	P-28	RescueMu, a Novel Mutagenesis and Gene Recovery Tool in Transgenic Maize and Wheat ***Manish N. Raizada,*** *University of Guelph*

10:00 am – 10:30 am Coffee Break Foyer

GENE TRANSFER

SPONSOR: BIO-RAD LABORATORIES, INC., BTX, DIVISION OF GENETRONICS

Convener: Richard Heller, University of South Florida

10:30 am – 12:30 pm Vertebrate Symposium Jefferson F

(See abstracts pages 17-A to 18-A)

Gene transfer holds the promise of being a means to effectively treat a wide variety of human diseases. A tremendous effort has been put forth by numerous investigators to establish protocols for the efficient delivery of genes to cells. These protocols cover a variety of applications and include delivery in the in vitro, ex vivo, and in vivo settings. Currently employed gene delivery systems primarily use viruses, however, the use of non-viral delivery systems have recently been increasing. This session will explore recent advances in the use of gene transfer and will include the discussion of both viral and non-viral delivery protocols.

10:30		Introduction (R. Heller)
10:45	V-4	Cationic Lipid Based Gene Transfer ***Ronald, K. Scheule,*** *Genzyme Corporation*
11:10	V-5	Gene Gun Applications: In Vivo Gene Expression Regulated by Tissue-specific Promoters ***Michael T. S. Lin,*** *Jefferson Medical College*
11:35	V-6	Development of Gene Therapy for Hemophilia B: Gene Regulation In Vitro and In Vivo, and Gene Transfer Vector Systems ***Kotoku Kurachi,*** *University of Michigan*
12:00	V-7	Electrically Enhanced Delivery of Plasmid DNA ***Richard Heller,*** *University of South Florida*

TROPICAL PLANT TRANSFORMATION/TISSUE CULTURE

SPONSOR: THE SCOTTS COMPANY

Convener: Ebe Firoozabady, DNA Plant Technology Corporation
Carlos G. Borroto, Center for Engineering and Biotechnology

10:30 am – 12:30 pm Plant Symposium Mississippi

(See abstracts page 13-A)

Transformation of some of the tropical crops has been a challenge. Recently, some advances have been made for transformation as well as introduction of some useful genes into important tropical crops. This session explores the recent development on several important tropical crops including sugarcane, sweet potato, cassava, mango, banana, and avocado. Also, strategies for use of transgenic plants for food security in tropical developing countries will be discussed.

Wednesday, June 20

10:30 Introduction (E. Firoozabady)
10:40 P-29 Strategies for Use of Transgenic Plants for Food Security in Tropical Third World Countries: Transgenic Sugarcane and Another Crop as Examples
Carlos G. Borroto, *Center for Engineering and Biotechnology*
11:30 P-30 Importance, Status, and Limitations of Cassava Transformation
Claude M. Fauquet, *The Donald Danforth Plant Science Center*
12:00 P-31 Genetic Transformation of Some Tropical Species
Miguel A. Gomez Lim, *CINVESTAV-Irapuato*

DISEASE RESISTANCE

Moderator: Nigel J. Taylor, Danforth Plant Science Center
Martin Steinau, Kansas State University

1:00 pm – 2:30 pm Plant Contributed Paper Session (See abstracts pages 25-A to 26-A) Jefferson A & B

1:00 P-1012 Constitutive Expression of an Endogenous Antifungal Protein Alpha-hordothionin in Transgenic Barley
Jianming Fu, *University of Wisconsin-Madison, P. Sathish, M. L. Federico, H. F. Kaeppler, and R. Skadsen*
1:15 P-1013 Expression of Maize *Rp1-D* Rust Resistance Gene in Transgenic Maize and Wheat
Martin Steinau, *Kansas State University, S. H. Hulbert, and H. N. Trick*
1:30 P-1014 Transformation of Peanut with Truncated Nucleocapsid Protein Gene of Tomato Spotted Wilt Virus Gene in Cultivated Peanut (*Aarachis hypogaea* L.) Using Particle Bombardment
Hongyu Yang, *University of Georgia, H. Pappu, and P. Ozias-Akins*
1:45 P-1015 Production of Fertile Transgenic Soybeans with Putative Enhanced Disease Resistance
Wojciech J. Ornatowski, *Kansas State University, W. Schapaugh, S. Muthukrishnan, T. C. Todd, and H. N. Trick*
2:00 P-1016 Transformation with a Pathogen-inducible Stilbene Synthase Gene for Increased Fungal Resistance in Papaya
Judy Y. Zhu, *Hawaii Agriculture Research Center, C. S. Tang, M. Fitch, and P. Moore*
2:15 P-1017 Transgenic Cassava for Resistance to African Cassava Mosaic Disease
Nigel J. Taylor, *Danforth Plant Science Center, and C. M. Fauquet*

TISSUE CULTURE AND REGENERATION

Moderator: David Songstad, Monsanto Company
Harold Trick, Kansas State University

2:45 pm – 5:00 pm Plant Contributed Paper Session (See abstracts pages 26-A to 28-A) Jefferson A & B

2:45 P-1018 A Study on the Polyamine Level During Somatic Embryogenesis Development in *Vitis vinifera*
Lucia Martinelli, *Institute Agrario Provinciale, D. Bertoldi, A. Tassoni, E. Candioli, I. Gribaudo, and N. Bagni*

3:00	P-1019	Cre/lox Mediated Marker Gene Excision in Transgenic Crop Plants ***Larry Gilbertson,*** *Monsanto Company, P. Addae, C. L. Armstrong, N. Bernabe, J. Ekena, G. Keithly, M. Neuman, V. Peschke, M. Petersen, S. B. Subbarao, W. Zhang, and K. Barton*
3:15	P-1020	In Vitro Regeneration of *Artemisia judaica* L. (Compositae) Via Shoot Organogenesis and Somatic Embryogenesis ***Skye S. B. Campbell,*** *University of Guelph, M. A. El-Demerdash, and P. K. Saxena*
3:30	P-1021	A Role for Serotonin and Melatonin in Plant Morphogenesis ***Susan J. Murch,*** *University of Guelph, and P. K. Saxena*
3:45	P-1022	Induction of Somatic Embryogenesis and Shoot Organogenesis on Thin Cell Layers of AfricanViolet (*Saintpaulia ionantha*) ***Jerrin M. R. Victor,*** *University of Guelph, S. J. Murch, and P. K. Saxena*
4:00	P-1023	Cryopreservation of Plumular Explants of Coconut (*Cocos nucifera* L.) ***Paul T. Lynch,*** *University of Derby, R. Hornung, and R. Domas*
4:15	P-1024	Changes in K, Mg, and Ca Levels in Embryogenic and Non-embryogenic Citrus Callus Subjected to Two Carbohydrate Sources for Somatic Embryogenesis Expression ***Adriana P. M. Rodriguez,*** *CENA/USP, S. C. C. Arruda, M. A. Z. Arruda, and B. M. J. Mendes*
4:30	P-1025	Using Tissue Culture to Generate *Phragmites*-blocking Wetland Plants ***Jiangbo Wang,*** *University of Delaware, J. L. Gallagher, and D. M. Seliskar*
4:45	P-1026	Ethylenediurea (EDU) and the Desiccation Effects of High Concentrations of Ozone on the Jade Plant (*Crassula argentea*) ***Cyril E. Broderick, Sr.,*** *Delaware State University, and G. A. Jones, III*

Joint and Invertebrate Posters

SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
8:00am-9:00pm	8:00am-9:00pm	10:00am-3:00pm

Posters mounted Saturday, June 16, 3:00pm-7:00pm.
Posters must be removed from Exhibit Hall by 6:00pm, June 19.
Authors will be present at their posters the following days and times:

SATURDAY, JUNE 16	SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	2:15pm – 2:45 pm	2:15pm – 2:45 pm	2:15pm – 2:30 pm

SECONDARY METABOLISM (INTERACTIVE)

JP-2000 In Vitro Propagation and Quantification of Rotenoids in Callus of Derris sp
José Eduardo Pinto, B.P., *UFLA/DAG, H. E. O. Conçeicão, N. E. A. Castro, E. J. A. Santiago, and O. A. Lameira*

JP-2001 Extraction and Detection of Kavapyrones from In Vitro Cultures of Kava (*Piper methysticum* Foster)
Hideka Kobayashi, *University of Illinois, M. A. L. Smith, M. Gawienowski, and D. Briskin*

JP-2002 Light Does Not Regulate All Steps in the Mevalonate Independent Pathway of Terpenoid Biosynthesis
Frederic F. Souret, *Worcester Polytechnic Institute, P. Weathers, and K. Wobbe*

JT-2003 In Vitro Effects of Semipure Protease Inhibitor Fractions from Edible Seeds on Malignant Cell Survival
Teresa Garcia-Gasca, *Universidad Autonoma de Queretaro, L. A. Salazar-Olivo, E. Mendiola-Olaya, C. Aguirre, and A. Blanco-Labra*

JT-2004 The Protection Effect of Fermented Products and Food Extracts Against Hydrogen Peroxide-induced Cytotoxicity
Jian-Chyi Chen, *Food Industry Research and Development Institute, Y.-H. Wei, R.-Z. Xie, S.-W. Wang, and S.-M. Hwang*

INVERTEBRATE POSTER

I-2000 Scanning Electron Microscopy of Midgut Epithelial Cells from *Dendroctonus valens* (Coleoptera:Scolytidae) Maintained *In Vitro*
Laura Sanchez, *Escuela Nacional de Ciencias Biolócigas-IPN, J. L. Andrade, M. E. Sánchez, R. Cisneros, and G. Zúñiga*

Plant Posters

SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
8:00am-9:00pm	8:00am-9:00pm	10:00am-3:00pm

Posters mounted Saturday, June 16, 3:00pm-7:00pm.
Posters must be removed from Exhibit Hall by 6:00pm, June 19.
Authors will be present at their posters the following days and times:

SATURDAY, JUNE 16	SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	2:15pm – 2:45 pm	2:15pm – 2:45 pm	2:15pm – 2:30 pm

DISEASE RESISTANCE (INTERACTIVE)

P-2000 Somatic Hybrids of *Solanum tuberosum* cv. Desiree and *S. chacoense* Bitt: A Baseline for Disease Resistance in Potato
Bushra Sadia, *University of Nottingham, P. Anthony, J. B. Power, K. C. Lowe, and M. R. Davey*

P-2001 Introduction of Sweetpotato Feathery Mottle Virus-coat Protein Gene into US and South African Sweetpotato Varieties via Agrobacterium tumefaciens
Chantal L. Daniels, *Tuskegee University, M. Egnin, and C. S. Prakash*

P-2002 Regenerants Derived from Leaf Explants of Several Strawberry Cultivars, Exhibit Increased Levels of Resistance to the Fungal Pathogen *Colletotrichum acutatum*
Freddi A. Hammerschlag, *USDA ARS Fruit Laboratory, S. Garces, M. Koch-Dean, J. Maas, and B. Smith*

P-2003 Constitutive Expression of Scab-inducible Genes for Enhancing Disease Resistance in Wheat
Ajith Anand, *Kansas State University, W. L. Li, N. Sakthivel, S. Krishnaveni, S. Muthukrishnan, B. S. Gill, and H. N. Trick*

DICOT TRANSFORMATION (INTERACTIVE)

P-2004 Optimization of Growth and Particle Bombardment-mediated Transformation of Embryonic Soybean Tissue, Maintained on a Semi-solid Medium
John J. Finer, *The Ohio State University, and A. J. Staron*

P-2005 Elevated Agar Concentration in the Co-cultivation Medium Considerably Improves Efficiency of Agrobacterium-mediated Transformation of Tomato
Sergei Krasnyanski, *University of Illinois, and S. S. Korban*

P-2006 Activation of Non-autonomous Maize Transposable Element, *Dissociation (Ds)*, by Ac-transposase in Carrot
Ahmet Ipek, *University of Wisconsin, and P. W. Simon*

P-2007 Regeneration of Transgenics of Picea glauca, P. Mariana, and P. abies After Co-cultivation of Embryogenic Tissue with Agrobacterium tumefaciens
Gervais Pelletier, *Laurentian Forestry Centre, K. Klimaszewska, D. Lachance, and A. Seguin*

P-2008 Optimizing the Transformation Efficiency for Flax
Kerry Ward, *Agriculture and Agri-Food Canada, and M. C. Jordan*

Plant Posters

DICOT TRANSFORMATION

P-2009 In Vitro Bioassay of Bt Toxin Expression in a Transgenic Cotton Callus Derived from a Non-regenerable Host Genotype
Benjamin Steinitz, *A. R. O.-The Volcani Center, Y. Gafni, Y. Cohen, S. Levski, Y. Tabib, and A. Navon*

P-2010 Early Senescence and Change of Sugar Composition Caused by Expression of a Carrot Acid Soluble Invertase in Tobacco (*Nicotiana tabacum* L.)
Yuan-Yeu Frank Yau, *University of Wisconsin-Madison, and P. W. Simon*

P-2011 Enhancement of Somatic Embryogenesis by Tryptophan in West African Cassava Cultivars
Rachelle N. N. Kokora, *Danforth Plant Science Center, N. J. Taylor, and C. M. Fauquet*

P-2012 Field Performance of Transgenic 'High Protein' Sweetpotatoes (*Ipomoea batatas* L., PI 318846-3) Show No Yield or Phenotypic Cost of an Extra Gene
Marceline Egnin, *Tuskegee University, C. L. Daniels, C. S. Prakash, L. Urban, T. Zimmerman, S. Crossman, and J. Jaynes*

P-2013 Transformation of Ethylene-response-sensor (ERS) Mutant Gene in Broccoli (*Brassica oleracea var. italica*) by *Agrobacterium tumefaciens*
Long-Fang O. Chen, *Institute of Botany, Academia Sinica, J. Y. Huang, H. H. Chen, and J. F. Shaw*

P-2040 Development of Plant Regeneration and Genetic Transformation in the Papveraceae for the Metabolic Engineering of Benzylisoquinoline Alkaloids
Sang-Un Park, *University of Calgary, and P. J. Facchini*

MONOCOT TRANSFORMATION (INTERACTIVE)

P-2014 Switchgrass Transformation by Microprojectile Bombardment with pAHC25 a GUS-BAR Construct
Judith K. McDaniel, *University of Tennessee, Z. Tomaszewski, V. Rudas, and B. V. Conger*

P-2015 Genetic Transformation of Switchgrass Mediated by *Agrobacterium tumefaciens*
Bob V. Conger, *University of Tennessee, and M. N. Somleva*

P-2016 Transformation Process Exacerbates Cytological Variation in Transgenic Grass and Cereal Plants
Hae-Woon Choi, *University of California – Berkeley, P. G. Lemaux, and M.-J. Cho*

P-2017 Use of Cyanamide Hydratase Gene as a Selectable Marker for the Transformation of Sorghum
Jayaraj Jayaraman, *Kansas State University, H. Yi, A. Anand, T. Weeks, G. H. Liang, and S. Muthukrishnan*

P-2018 An Efficient System for Transformation and Plant Regeneration of Sorghum Using Highly Regenerative, Green Tissues
Myeong-Je Cho, *University of California – Berkeley, and P. G. Lemaux*

P-2019 High-frequency Transformation of Rice (*Oryza sativa* L.) via Microprojectile Bombardment of Mature Seed-derived Highly Regenerative Tissues
Myeong-Je Cho, *University of California – Berkeley, H. Yano, D. Okamoto, V. K. Le, K. L. Newcomb, B. B. Buchanan, and P. G. Lemaux*

P-2020 Long-term Stability of Transgene Expression Driven by Barley Endosperm-specific Hordein Promoters in Transgenic Barley (*Hordeum vulgare* L)
Hae-Woon Choi, *University of California – Berkeley, P. G. Lemaux, and M.-J. Cho*

IN VITRO TOOLS AND TECHNIQUES

P-2021 Defining Optimal Storage Conditions for Cotton Tissues Prior to Ovule Culture
Barbara A. Triplett, *USDA-ARS, and D. S. Johnson*

P-2022 Cryopreservation of Embryogenic Avocado (*Persea americana* Mill.) Cultures
Darda Efendi, University of Florida, *R. E. Litz, and F. Al-Oraini*

P-2023 Shipping Procedures for Plant Tissue Cultures
Barbara M. Reed, *USDA-ARS National Clonal Germplasm Repository, C. L. Paynter, and B. Bartlett*

P-2024 Overcoming of Interspecies Incompatibility in the Solanaceaous Genera *Nicotiana* and *Capsicum* via In Vitro Techniques
Violeta M. Nikova, *Bulgarian Academy of Sciences, R. D. Vladova, A. C. Petkova, and A. Iancheva*

P-2041 Development of Intergeneric Hybrids in Crop Brassicas via Embryo Rescue and Somatic Hybridization
G. Ravi Kumar, *Indian Agricultural Research Institute, S. R. Bhat, Shyam Prakash, and V. L. Chopra*

IN VITRO TISSUE CULTURE, MICROPROPAGATION, AND SOMATIC EMBRYOGENESIS

P-2025 *In Vitro* Culture of Sea Thrift (*Armeria maritima*)
Paul T. Lynch, *University of Derby, L. Brewin, A. Mehra, and M. E. Farago*

P-2026 A New Approach for In Vitro Regeneration of *Phaseolus Vulgaris*
Magfrat Muminova, *Institute of Genetics-Uzbekistan, M. Nasretdinova, and S. Djataev*

P-2027 Development of Shoot Culture Protocols for Eastern Black Walnut (*Juglans nigra*)
Michael J. Bosela, *USDA Forest Service, and C. H. Micheler*

P-2028 Plant Regeneration from Sugarcane Seed-derived Callus
Chengalrayan Kudithipudi, *University of Florida, A. Abouzid and M. Gallo-Meagher*

P-2029 Factors Affecting Micropropagation of *Asimina tetramera,* an Endangered Florida Scrub Species
John R. Clark, *Cincinnati Zoo and Botanical Garden, and V. C. Pence*

P-2030 An Alternative Propagation Method of *Bergenia ligulata* Through Leaf Culture
Prakash Raj Malla, *Tribhuvan University, and S. Malla*

P-2031 Somatic Embryogenesis, Secondary Somatic Embryogenesis, and Shoot Organogenesis in *Rosa hybrida* and *Rosa chinensis* minima
Xiangqian Li, *University of Illinois at Champaign-Urbana, S. F. Krasnyanski, and S. S. Korban*

P-2032 Maturation and Germination of Somatic Embryos from Three Distinct Cultivars of Rose
Kathryn K. Kamo, *USDA, J. Castillon, and B. Jones*

P-2033 Histology and Scanning Electron Microscopy of Somatic Embryo Development in Grapevine
S. Jayasankar, *MREC-University of Florida, D. J. Gray, Z. Li, and B. R. Bondada*

Plant Posters

SILENT ABSTRACTS

P-2034 Influence of UV Rays on Pepper (*Capsicum annum* L.) Cultivated In Vitro
Ivanka Y. Kozareva, *University of Florida, N. Zagorska, V. Sotirova, S. Daskalov, B. Dimitrov, V. Lapshin, and R. Butenko*

P-2035 Gene Introduction Method Affects Transgene Expression in Chrysanthemum (*Dendranthema grandiflora*)
Jaime Alberto Teixeira Da Silva, *Kagawa University, and S. Fukai*

P-2036 Comparative Effect of BAP and TDZ on Multiplication of Micropropagated Saffron (*Crocus sativus* L.) Corms
Jose A. Fernández, *University of Castilla-La Mancha, S. Blázquez, A. Piqueras, and C. Rubio*

P-2037 Micropropagation of Triploid Crossandra
Ganga Mathian, *Tamil Nadu Agricultural University, N. Chezhiyan, and K. A. Shanmugasundaram*

P-2038 Effect of the Substituted Chromosomes Upon Developmental Processes In Vitro in 20 Wheat Lines
Vasil K. Chardakov, *Bulgarian Academy of Sciences, A. Dryanova, N. Tyankova, N. Zagorska, and B. Dimitroff*

P-2039 Changes in Polyamine Metabolism During the Acclimatization of Micropropagated *Populus* Plants
Jose L. Casas-Martinez, *Unversidad de Alicante, M. Cortina, M. D. Serna, and A. Piqueras*

Vertebrate / Toxicology Posters

SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
8:00am-9:00pm	8:00am-9:00pm	10:00am-3:00pm

Posters mounted Saturday, June 16, 3:00pm-7:00pm.
Posters must be removed from Exhibit Hall by 6:00pm, June 19.
Authors will be present at their posters the following days and times:

SATURDAY, JUNE 16	SUNDAY, JUNE 17	MONDAY, JUNE 18	TUESDAY, JUNE 19
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
7:30 pm – 8:30 pm	2:15pm – 2:45 pm	2:15pm – 2:45 pm	2:15pm – 2:30 pm

IN VITRO TOXICOLOGY (INTERACTIVE)

VT-2000 Establishment of a Human Hepatoma Cell Line HLE/2E1, Suitable for Detection of P450 2E1-Related Cytotoxicity
Isao Nozaki, *Okayama University Medical School, and M. Namba*

VT-2001 Study of Embryonic Ploidy: A Probable Embryo Model
Miriam Soledad Kundt, *National Atomic Energy Commission, and R. L. Cabrini*

VT-2002 Three-dimensional Transgenic Model for Genotoxic Assessment Using Macroporous Cultispheres
Denise N. Fraga, *University of Notre Dame, J. A. Jordan, and S. R. Gonda*

VT-2003 The Effects of Exogenous Hormones on the Cytotoxicity of Chemically Modified Tetracyclines on LNCaP Human Prostate Tumor Cells
Heather L. Sawka, *State University of New York at Stony Brook, S. R. Simon, and E. J. Roemer*

VT-2004 Cytotoxic Effects of Raloxifene on Mouse and Human Cancer Cell Lines
Shyamal K. Majumdar, *Lafayette College, M. C. Davis, and K. Ouchi*

VT-2005 Assessing Tissue Specific Toxicity of Chemopreventive Agents in Cultures from Normal Human Tissues
Eugene L. Elmore, *University of California-Irvine, T.-T. Luc, G. J. Kelloff, V. E. Steele, and J. L. Redpath*

PART 1 – EVALUATION OF CRYOPRESERVATION TECHNIQUES (INTERACTIVE)
PART 2 – HUMAN EPIDERMAL KERATINOCYTES (INTERACTIVE)

VT-2006 Evaluation of Cell Viability During Cryopreservation Using Cell Culture Medium Versus Low-temperature Storage Solutions
Lia H. Campbell, *Organ Recovery Systems, M. J. Taylor, and K. G. M. Brockbank*

VT-2007 Enhanced Hypothermic Preservation of Human Renal Cells and Human Epidermal Keratinocytes
Aby J. Mathew, *State University of New York at Binghamton, J. G. Baust, and R. G. Van Buskirk*

VT-2008 Comparison of Cell Viability Using Unisol and Other Preservation Solutions During Hypothermic Storage
Lia H. Campbell, *Organ Recovery Systems, M. J. Taylor, and K. G. M. Brockbank*

VT-2009 Cultured Epithelial Skin Grafts
Frederick O. Cope, *Hy-Gene Biomedical Corporation, J. Wille, N. Swanson, M. Pittelkow, and J. Burdge*

VT-2010 Interleukin-8 (IL-8) as a Biomarker for Vesicant Agent-induced Cytotoxicity in Normal and Immortalized Human Keratinocytes
Raymond Vazquez, *USAMRICD, M. R. Nelson, J. J. Guzman, C. M. Corun, M. Steinberg, and C. M. Arroyo*

VT-2011 Human Epidermal Keratinocytes Exposed In Vitro to the Vesicating Agent Sulfur Mustard Express Markers of Apoptosis and Inflammation
William J. Smith, *US Army Medical Research Institute of Chemical Defense, E. W. Nealley, O. E. Clark, and F. M. Cowan*

OCULAR MODELS (INTERACTIVE)

VT-2012 The Identification and Quantification of Z-DNA in Congenital Cataracts
Claude E. Gagna, *New York Institute of Technology, C. Philip, and W. C. Lambert*

VT-2013 Characterization of a Human Conjunctival Epithelial Cell Line
Yolanda Diebold, *IOBA-University of Valladolid, M. Calonge, R. M. Corrales, A. Enríquez de Salamanca, M. V. Sáez, and E. Pestaña*

VT-2014 Growth of Human Corneal Epithelial and Stromal Fibroblast Cells in Serum-free Media
Sim F. Webb, *University of East Anglia*

SILENT ABSTRACTS

VT-2015 Cryopreservation of Immature Bovine Oocytes Treated with EGTA
Laura Simonetti, *Universidad Nacional de Lomas de Zamora, and M. R. Blanco*

VT-2016 3D Distribution of erb-B1 Receptors on Rat Colonocytes in Primary Cultures
Bertrand A. Kaeffer, *Institut National Recherche, A. Trubuil, C. Kervrann, M.-F. Devaux, and C. Cherbut*

VT-2017 Preliminary Ultrastructural Data on the Innervation of the Interstitial Cells During the Differentiation of the Chick Ovary Cultured with LH or hCG
Rodolfo E. Avila, *University of Cordoba-Argentine, M. E. Samar, R. Ferraris, F. J. Esteban, J. A. Pedrosa, and M. A. Peinado*

VT-2018 The Effect of Protease Inhibitors on Triglyceride Synthesis and Insulin Signaling in L6 Myotubes
Ralph J. Germinario, *Lady Davis Institute, and S. P. Colby-Germinario*

2001 Congress on In Vitro Biology

Exhibitor List

As of April 2, 2001

Ambion, Inc.
Austin, TX

AMS Midlands
Medical Technologies
Mediquip Parts Plus
St. Louis, MO

Bio-Rad Labs
Hercules, CA

BTX, Div. of Gentrn
San Diego, CA

CABI Publishing
Wallingford, Oxon, UK

Conviron
Boiling Springs, SC

Cook Biotech, Inc.
West Lafayette, IN

Guava Technologies
Burlingame, CA

Harvard Bioscience
Holliston, MA

Innovative Cell Technologies
San Diego, CA

InvitroGen - Life Technologies
Rockville, MD

Monsanto Company
St. Louis, MO

Percival Scientific, Inc.
Boone, IA

Phyto Technology Laboratories
Overland Park, KS

Sigma-Aldrich Corporation
St. Louis, MO

ThermoForma Scientific
Marietta, OH

VWR Scientific Products
St. Louis, MO

St. Louis 200
The Congress on In Vitro Biology
June 16 - 20, 2001 — St. Louis, Missouri

PS-1

Opportunities and Challenges in Plant Biology to Benefit Health and Sustainability. ROGER N. BEACHY, J. Skolnick, and K. Schubert. Donald Danforth Plant Science Center, St. Louis, MO 63105. Email: rnbeachy@danforthcenter.org

Recently scientists determined the sequence of the genomes of *Arabidopsis thaliana* and *Oryza sativa* (rice) that will make possible significant breakthroughs in food production, nutrition, and sustainability. Advances in biotechnology have produced food crops that require less chemical inputs to sustain high yields, and crops that promise increased levels of *beta*-carotene and improved health for consumers. The way forward to making the most of these and other recent scientific advances is to create multi-disciplinary teams of scientists that address and solve important and useful questions. Bioinformatics and computational biology are combining to enable researchers to predict the function of genes, while structural biology, biochemistry and cell biology work to determine the function of the gene. Molecular genetics and transformation technologies can determine the role of the gene in phenotyping of genetic function. These advances not withstanding, it remains to the scientist, in collaboration with societal expectations, to use the knowledge in responsible and useful ways. At the very least, these advances can lead to a more sustainable, highly productive agriculture system. At the very best it can lead to improved nutrition, health and well being of human beings and animals. However, the opportunities provided by scientific advances will progress only as far as the public permits and embraces the products. It remains the charge of the scientist to keep public officials, regulatory officials and consumers aware and knowledgeable of the promise and products of the new genetics. If that effort drags or fails, the world will not reap the benefits of our work.

J-2

Structural and Functional Analysis of a Maize Centromere. J.A. BIRCHLER. Division of Biological Sciences, University of Missouri, Columbia, MO 65211. E-mail: BirchlerJ@Missouri.edu

Over the past decade our laboratory has been studying the supernumerary B chromosome of maize to determine the minimum size of a functional centromere and of a functional chromosome. The centromere of the B chromosome contains a specific sequence repeat that allows one to follow changes in its structure over the background of the other centromeres. A natural process referred to as misdivision of the centromere was used to divide the centromere into smaller and smaller units followed by tests of meiotic transmission. The full sized centromere is approximately 9 megabases in size, but derivatives that have ungone multiple misdivisions have been recovered that are only approximately 90 kb in size. Centromeres of this size have reasonably good mitotic transmission but are significantly impaired for passage through meiosis. In addition, minichromosomes have been derived from the B chromosome that consist of little more than the centromere. Their inheritance has been examined in order to learn the minimum size of a chromosome that can be perpetuated from one generation to the next. Most of the minichromosomes suffer significant loss during meiosis. Fragments of the small misdivision derived centromeres have been cloned in bacterial artificial chromosome vectors and subjected to sequence analysis. They have also been used as candidate sequences for centromere function in trials of maize artificial chromosome vectors. Transformation of centromere sequences back into plants will potentially allow a functional test and may lead to the development of artificial chromosome manipulations in biotechnology.

J-3

Engineering Large Mammalian Artificial Episomal Chromosomes. JONATHAN BLACK and Jean-Michel Vos. Lineberger Comprehensive Cancer Center School of Medicine, University of North Carolina at Chapel Hill, NC 27599-7295. E-mail: jablack@med.unc.edu

Mammalian artificial chromosomes (MAC) offer the capacity for introducing large DNA fragments into both differentiated and embryonic stem cells. Two general strategies are being developed to engineer large MACs with *de novo* function: (1) *in vitro* "bottom-up" chimeric MAC (buMAC) cloning by enzymatic ligation of individual MAC components followed by propagation in bacteria or yeast; and (2) *in situ* "top-down" MAC (tdMAC) assembly by co-introduction of various MAC elements into a mammalian tissue culture cell and use of it as a "foster parental" donor. Although both strategies generate stable MACs, each offers distinct advantages for therapeutic vector development. The organizational compactness and versatility of buMACs make them ideally suited for somatic-based human gene therapies while the careful and directed targeted-chromosome-fragmented tdMACs can be capitalized on to generate husbandry transgenic animals expressing therapeutic genes. As a proof-of-concept, our laboratory designed a novel chimeric buMAC equipped with the oriP/EBNA1 paradigm of the Type 4 latently episomal Epstein-Barr virus cloned into a pBELOBAC11 backbone (EBV/BAC). This EBV/BAC-based vector, later equipped with the entire 185 Kb human beta-globin loci, is mitotically stable in cultured human and mouse cells without selection for 3 months as demonstrated by Southern blot. In addition, transgene expression of the human beta-globin protein is detectable by RT-PCR. We believe this EBV/BAC-based vector offers a novel and attractive alternative for somatic-based gene therapy.

J-4

Development and Application of Artificial Chromosome Expression Systems (ACes). EDWARD L. PERKINS. Chromos Molecular Systems Inc., 8081 Lougheed Highway, Burnaby, BC V5A 1W9, CANADA. Email: www.chromos.com

Chromos Molecular Systems Inc. is engaged in the development and commercialization of Artificial Chromosome Expression Systems, or *ACes*, for use in the development of novel therapeutic products via ex vivo gene therapy and cell therapy applications, and the cellular and transgenic production of therapeutically relevant proteins. In addition, the technology has the potential to facilitate functional studies of complex genomes. Chromos has engineered a variety of novel *ACes*, s expressing therapeutic gene targets and various markers. Data will be presented demonstrating the construction and engineering of *ACes*,, production and delivery of ACes and stable transmission of engineered *ACes*.

J-5

Gene Discovery in Plants by Activation Tagging. HELENA MATHEWS. Exelixis Plant Sciences, Inc., 16160 SW Upper Boones Ferry Road, Portland, OR 97224-7744. Email: hmathews@exelixis.com

The plant genome is comprised of thousands of genes that control the normal growth, physiology, reproduction and disease resistance strategies of the plant. Understanding the functions of these genes is a major challenge for researchers, and a variety of forward and reverse genetic methods are being applied to this challenge. Large-scale insertional mutagenesis offers several significant features as a genomics platform. DNA insertions allow rapid functional analysis of phenotypes and the associated genes in a forward genomics research program. In addition, the indexed collection of insertion mutants creates basis for a robust program in reverse genomics by which one may analyze phenotypes associated with any given gene identified only by sequence. To take full advantage of the power of insertional mutagenesis approaches we have developed a high-throughput T-DNA mutagenesis program that incorporates transcriptional enhancers within the T-DNA vector. This system is known as Activation Tagging, or ACTTAG, and our platform is designed so that both dominant (gain-of-function) and recessive (loss-of-function) mutations can be quickly identified and the associated genes cloned. In addition, we have developed our ACTTAG platform with several plant species, including Arabidopsis, Micro-Tomato and rice. This diversity of species is necessary for a broad platform in trait identification, as each of these model systems has unique attributes required for the analysis of key plant processes. The creation of these ACTTAG mutant collections - in combination with state-of-art genomics capablities in DNA sequencing, informatics and transcriptional profiling - have created a powerful research and discovery platform.

J-6

Gene Expression Profiles Reveal Effector Pathways of Toxicants. H.K. HAMADEH. Laboratory of Molecular Carcinogenesis, Microarray Center, NIEHS, Research Triangle Park, NC 27709. E-mail: hamadeh@niehs.nih.gov

In the study of the impact of the environment on human health, there are formidable problems in relating the findings of chemical and environmental adverse effects in animal and other models to potential human risk. The necessity to use a variety of assumptions, including cross-species and low-dose extrapolations, coupled with an incomplete understanding of the mechanism of action of toxicants limits the scientific basis of risk assessment. Advances in genomics and new technologies, including cDNA microarrays, provide the capacity to measure global alterations of gene expression as a consequence of environmental perturbations. Traditional mechanistic studies in chemically exposed animals associate toxic and pathological changes with a very limited number of genes, proteins, or pathways. The application of gene expression profiling technology to examine multiple genes and multiple signaling pathways simultaneously promises a significant advance in understanding toxic mechanisms. The principal hypothesis underlying a toxicogenomic strategy is that chemical-specific patterns of altered gene expression will be revealed using high-density microarray analysis of tissues from treated organisms. Analyses of these patterns may reveal classes of toxicants and provide mechanistic insights. Patterns of gene expression were analyzed in liver tissue derived from chemically exposed Sprague-Dawley rats using cDNA microarrays. Clustering analysis and statistical correlation revealed that while gene expression profiles produced in animals treated with different agents from a common class of compounds, peroxisome proliferators (clofibrate, Wyeth 14,643 and gemfibrozil), were similar, a very distinct gene expression profile was produced using a compound from a completely distinct class, barbiturates (phenobarbital). In addition, the gene expression profiles not only validated previously reported mechanistic changes associated with the metabolism, pharmacology, and toxicology of these chemicals, but also revealed a substantial number of additional signaling events associated with exposure that provide new insight into potential mechanisms of chemical-mediated hepatotoxicity.

J-7

Proteomics: The View from a 2D Electrophoresis Service Lab. N.C. KENDRICK. Kendrick Labs Inc., 1202 Ann St., Madison, WI 53713. E-mail: 2d@kendricklabs.com

Kendrick Labs has been performing 2D protein electrophoresis as a commercial service since 1981. In the year 2000 we ran over 2300 2D gels for over 300 scientists in academia and industry. In this talk the methodology used at Kendrick Labs will be described. A variety of 2D applications will be presented, such as monitoring protein production during bacteria fermentation, and characterizing phosphorylated proteins in eukaryotic cells. The most important problems from our viewpoint will be discussed including some with protein identification by mass spectrometry.

J-8

Application of cDNA Microarray to Minute Amount of Biological Samples. K.K. WONG and R.S. Cheng. Molecular Biosciences Department, Pacific Northwest National Laboratory, Richland, WA 99352. S.C. Mok. Laboratory of Gynecologic Oncology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115. E-mail: kk.wong@pnl.gov

The use of cDNA microarray allows an investigator to measure the expression of thousands of genes simultaneously in a specific biological sample. By coupling with bioinformatic analysis, it is possible to assign potential functions to both known and unknown genes. One of the limitations of microarray technology is the requirement of a significant amount of total RNA for a single hybridization experiment. As a result, it is challenging to apply the microarray technology when the sample is in a limited amount, such as in primary cell culture, minute amount of tissue from small animals or laser-captured microdissected cells. Recently, several methods have been developed to tackle this problem. Some methods are based on the amplification of minute amount of total RNA by PCR-based method or *in vitro* RNA transcription, while other methods are based on signal amplification after hybridization. In this presentation, we will discuss the application of TSA amplification and 3DNA probe for gene expression profiling of primary human ovarian epithelial cells in one case, and uteri dissected from ovarectomized mice in another. We were able to obtain about 20 μg of total RNA from each batch of primary ovarian epithelial cells, while we can only obtain about 10 μg total RNA from uteri, pooled from 3 ovarectomized mice. In our studies, we were able to identify differentially expressed genes from 2-3 μg of total RNA extracted from primary human ovarian epithelial cells. Similarly, we were also able to follow the gene expression changes in the uteri of ovarectomized mice during treatment with 17-beta-estradiol for 3 days. The differential expression of several interested genes were further confirmed by real-time quantitative PCR.

J-9

From How Many Different Cells Can a Plant Make an Embryo? K. BOUTILIER. Plant Research International, Postbus 16, 6700 AA Wageningen, THE NETHERLANDS. E-mail: k.a.boutilier@ant.wag-ur.nl

Higher plants display the remarkable characteristic of totipotency, that is, the ability of a cell, given the appropriate stimuli and growth conditions, to regenerate a complete plant. One example of totipotency in plants is the formation of embryos in the absence of fertilization. In sexually reproducing plants, the megaspore mother cell undergoes meiotic/mitotic divisions to produce a haploid eight-nucleate embryo sac. Fertilisation of the egg cell and two central cell nuclei of the embryo sac by the haploid male gametes of the pollen grain produce, respectively, the diploid embryo and the triploid endosperm (the nutritive tissue of the seed). Apomictic plants differ from sexual in plants in that egg cell fertilisation is not a prerequisite for embryo formation. Three major mechanisms of apomixis have been observed, in which embryos either develop by parthenogenesis of one of the cells of an unreduced embryo sac or directly from the surrounding sporophytic tissue. Asexually-derived embryos can also be induced to form in culture from many gametophytic and somatic plant tissues by treating them with plant growth regulators or by a simple stress treatment. The research in our group is directed toward understanding the molecular processes that control the initiation of the embryonic pathway in plants. For this, three model systems are used: *Arabidopsis thaliana*-zygotic embryogenesis; *Arabis holboelli* - apomixis; and *Brassica napus*- in vitro microspore embryogenesis. Research during the last few years, mainly using mutagenesis and differential gene expression analysis, has led to the identification of mutants or genes believed to control the initiation of embryo development in plants. Chief among these is *BABY BOOM*, an AP2 domain transcription factor gene that promotes the spontaneous conversion of vegetative tissues into embryos. During my talk I will provide an overview of the different ways in which plants initiate embryo development, the model systems we use to study this process, and our efforts to identify key regulatory genes controlling the entry into the embryonic pathways in plants.

J-10

Molecular Control of Muscle and Heart Development During *Drosophila* Embryogenesis. ALAN M. MICHELSON[1], Marc S. Halfon[1], Ana Carmena[2], Fernando Jiménez[3], and Mary K. Baylies[2]. [1]Howard Hughes Medical Institute and Brigham & Women's Hospital, Boston, MA 02115; [2]Memorial Sloan-Kettering Cancer Institute, NY, NY 10021; [3]Univ. Miguel Hernandez, Alicante, SPAIN. E-mail: michelson@rascal.med.harvard.edu.

A common theme in animal development is the progressive restriction of cell fates through the combined action of intrinsic and extrinsic factors. We have been examining the interplay of such factors during the specification of muscle and cardiac progenitors in the *Drosophila* embryo. Uncommitted mesodermal cells initially receive Wnt and BMP signals which together activate the expression of proteins that render them competent to respond to subsequent EGF and FGF signals. Ras pathway activation, mediated by EGF and FGF receptors, stimulates the formation of equivalence groups from which individual muscle and cardiac progenitors are singled out. The latter process involves an antagonistic interaction between persistent Ras activity and the inhibitory influence of the Notch pathway. Further complexity is manifest by the occurrence of a positive feedback loop which amplifies the inductive Ras signal, by the ability of Notch to block MAPK activation, and by synergy between Notch and an EGF receptor inhibitor. In addition, expression of the Notch ligand, Delta, is upregulated by Ras in the emerging progenitor, thereby providing a mechanism for spatially localizing the primary lateral inhibitory signal. Finally, integration of Wnt, BMP and Ras signals occurs in the nucleus such that transcription factors acting in each of these pathways converge to regulate a single progenitor identity gene enhancer. Two mesoderm-intrinsic transcription factors also function on this enhancer, insuring the generation of a tissue-specific response to otherwise pleiotropic extrinsic signals.

J-11

Characterization and Differentiation of Human Embryonic Stem Cells. M.K. CARPENTER, M.S. Inokuma, J. Denham, L. Rambhatla, P. Kundu, L. Huang, C. Xu, and C.P. Chiu. Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025. E-mail: mcarpenter@geron.com

Human embryonic stem (huES) cells generated from blastocysts can proliferate and maintain their pluripotency for over one year in vitro (Amit et al., 2000). In addition, these cells can be maintained without feeders and retain telomerase activity, appropriate surface markers and OCT-4 expression. In these conditions, the cells retain their capacity to form all three embryonic germ layers in vitro and in vivo. We have investigated the differentiation of huES cells into neural cells, cardiomyocytes, and hepatocyte-like cells. Using appropriate culture conditions, huES cells can differentiate into significantly enriched populations, with 60-80% of the cells expressing specific markers, such as NCAM or albumin. The ability to grow phenotypically and karyotypically stable huES cells for extended periods of time allows the large scale production of these cells. In addition, the ability of the huES to generate neural, cardiomyocyte, and hepatocyte lineage indicates that huES cells may be an appropriate source for cell replacement therapies.

I-1

Hormonal Regulation of the Transcriptional Cascade Leading to Dopa Decarboxylase Expression. K. HIRUMA and L.M. Riddiford. Department of Zoology, University of Washington, Seattle, WA 98195-1800. Email: hiruma@u.washington.edu

During the last larval molt in *Manduca sexta*, the mRNAs for the two isoforms of EcR and USP switch and several transcription factors (MHR3, MHR4, and βFTZ-F1) are sequentially expressed with the changing ecdysteroid titer culminating in dopa decarboxylase (DDC) expression in the epidermis just before sclerotization occurs. MHR3 appears with the increase in 20-hydroxyecdysone (20E), MHR4 as the titer begins to decline and βFTZ-F1 when the titer is low. Culturing of day 2 4th instar epidermis with high 20E caused first the appearance of MHR3 at 3 hr, then MHR4 between 12 and 24 hr. Both are directly induced by 20E, but MHR3 requires a 20E-induced protein for full activation whereas MHR4 is suppressed by a 20E-induced protein. By contrast, βFTZ-F1 mRNA expression requires exposure to 20E followed by its removal as does DDC; both 20E and a 20E-induced protein are involved in inhibition of these 2 mRNAs. *Drosophila* βFTZ-F1 bound to the *Manduca* DDC promoter in gel shift assays, and the expression of βFTZ-F1 in *Manduca* GV1 cells activated the DDC promoter. Therefore, βFTZ-F1 is a positive activating factor for DDC. Experiments are in progress to determine whether MHR4 which also binds to the FTZ-F1 response element inhibits DDC expression. Supported by the USDA and NIH.

I-2

Non-steroidal Ecdysone Agonists: *In Vitro* Methods for Discovery and Use for Agriculture and Pharmaceutical Markets. T.S. DHADIALLA and G.R. Carlson. Ag. Discovery, Rohm and Haas Company, Spring House, PA 19477. E-mail: RSATSD@rohmhaas.com

While the discovery of the first non-steroidal bisacylhydrazine ecdysone agonist chemistry came about through biological testing in whole insect systems, the discovery of the commercial compounds in this class of chemistry was significantly enhanced through the use of cell free and cell based assays *in vitro*. Likewise, tremendous knowledge on the molecular basis of action of this class of chemistry has also come about through use of *in vitro* methods, ranging from cell free ecdysone receptor complex binding radiometric assays to examination of specific gene activation in insect tissues cultured *in vitro*. In this presentation, an overview of how *in vitro* assays and methods led to the discovery of the commercial non-steroidal bisacylhydrazine insecticides will be discussed. Additional assays for the discovery of new members of this class of chemistry with different properties will also be described. The potential use of members of this class of chemistry in regulating traits in plants and animal systems in the future will also be discussed.

I-3

Mode of Action, Specificity and Possible Resistance Mechanism of Non-steroidal Ecdysone Analogs. A. Retnakaran, Q. Feng, and B. Arif. Great Lakes Forestry Center, Sault Ste. Marie, On, CANADA P6A 5M7. Email: aretnak@nrcan.gc.ca

Non-steroidal ecdysone analogs, act at the molecular level in larvae similar to the molting hormone but unlike the native hormone are not cleared from the system and induce an incomplete, precocious molt that is lethal. Day 0 sixth instar larvae of the spruce budworm, *Choristoneura fumiferana*, upon ingesting such a compound, stop feeding and go into a precocious molt that is incomplete and stay frozen in this developmental state eventually dying of starvation and dessication. Similar to ecdysone, it binds to the receptor complex and transactivates the expression of a sequence of up-regulated genes. But unlike the native hormone which is cleared after this event, this agonist persists and as a result the down-regulated genes that are normally expressed in the absence of the hormone are not expressed. The toxicities of 4 agonists were tested on the spruce budworm and it correlated well with transactivation. We found that one such analog, Tebufenozide, was active on lepidopteran cells but inactive on dipteran cells and could be traced to an exclusion mechanism similar to an ABC transporter. Using several ABC transporter mutants of yeast we determined that PDR5 was responsible for the exclusion of Tebufenozide in yeast. Recently we discovered that, while younger instars are susceptible, the older instars of the white marked tussock moth (*Orgyia leucostigma*) are resistant to this compound. Upon ingesting Tebufenozide the larvae show head capsule slippage, remain moribund for a few days but then recover and molt into the subsequent instar suggesting a resistance mechanism possibly based on exclusion.

I-4

In Vitro Imaginal Disc Cultures as Bioassay for Ecdysone Action. G. SMAGGHE, B. Carton, L. Decombel, A. Heirman, and L. Tirry. Laboratory of Agrozoology, Ghent University, Ghent, BELGIUM. E-mail: GUY.SMAGGHE@RUG.AC.BE

Dibenzoylhydrazine ecdysone agonists are a new group of insect growth regulators (IGRs) leading specifically to premature, lethal larval molting in Lepidoptera. The molting hormone activity of methoxyfenozide (RH-2485), tebufenozide (RH-5992), halofenozide (RH-0345) and RH-5849 was measured using cultured imaginal wing discs from last-instar beet armyworms (*Spodoptera exigua* Hübner) and cotton leafworms (*Spodoptera littoralis* Boisduval). Methoxyfenozide was the most potent in terms of potency to induce disc evagination. In addition, we determined the activity of the ecdysone agonists to bind with the target receptors in whole discs in competition with [^{3}H]labeled ponasterone A as compared to the natural molting hormone 20-hydroxyecdysone. Interestingly, a linear regression was found between evagination-induction *in vitro* and toxicity against whole caterpillars for both pest insect species. This supports the utility of such imaginal disc bioassay for investigating and screening for putative ecdysone agonists.

I-5

Comparative Structure-Activity Relationship of Various Nonsteroidal Ecdysone Agonists Between In Vivo and In Vitro Assay Systems. Y. NAKAGAWA. Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, JAPAN. Email: NAKA@KAIS.KYOTO-U.AC.JP

Dibenzoylhydrazine analogs such as RH-5849 and tebufenozide are well known as nonsteroidal ecdysone agonists, and demonstrate the insecticidal activity. To date four analogs tebufenozide, methoxyfenozide, halofenozide, and chromafenozide have been registered as insecticides. The structure-activity relationships for the insecticidal activity are vary greately with species. The insecticidal activity of dibenzoylhydrazine analogs, containing various substituted benzene rings, against *Chilo suppressalis* was linearly correlated with their molting hormonal activity measured in the *Chilo* cultured integument assay system. The inhibition of the binding of tritiated ponasterone A by dibenzoylhydrazine analogs and six ecdysone analogs was measured against two intact insect cell lines, *Spodoptera* Sf-9 and *Drosophila* Kc. The binding activity measured against Sf-9 was nicely correlated with the molting hormonal activity against *Chilo* integument, suggesting that the structure-activity relationship profile is very similar between lepidopterous insects *Chilo* and *Spodoptera*. The profiles for the structure-activity relationship were, however, very different between these two insect cell lines. The binding activity measured in Kc cells was sensitive to the structural modification of steroidal ecdysones, but that for Sf-9 cells was sensitive to dibenzoylhydrazine structures.

I-6

Development of New Screening Systems for Hormonal Compounds Using Transformed Insect Cell Lines. L. SWEVERS (1), L. Kravariti (1), M. Ksenou-Kokoletsi (1), K. Sdralia (1), V. Mazomenos (1), N. Ragousis (2), and K. Iatrou (1). (1) Institute of Biology, NCSR Demokritos; and (2) Vioryl Chemical Co., Athens, GREECE. E-mail: swevers@mail.demokritos.gr

The cloning of hormone receptors combined with the identification of their intracellular transduction pathways has opened new possibilities for the development of cell-line based screening systems for (anti-)hormonal compounds. They are based on cell lines transformed for two genetic elements, (1) a cassette that drives the expression of a ligand-activated hormone receptor and (2) a reporter cassette that is under control of the transcription factor activated by the hormone or hormone receptor. Using this approach, different silkmoth (Bm5 cell)-derived cell lines were developed that are transformed for an ecdysone-responsive GFP reporter construct and screenings for ecdysone-like compounds in plant extracts were carried using these cell lines. Ecdysone mimetic compounds were identified in extracts from spinach and *Chenopodium* of which one was characterized by mass spectrometry as 20-hydroxy-ecdysone. Antagonistic substances were shown to be present in extracts from several Citrus species and their purification by HPLC is in progress. A similar rationale is currently applied to develop cell-line based screening systems for juvenile hormone (JH)-like compounds. Screening for JH-like compounds is based on the observation that JH causes multimerization of the Ultraspiracle (USP) nuclear receptor. The development of a two-hybrid assay in Bm5 cells, using the silkmoth homolog of USP, that is predicted to be activated by JH, is currently in progress.

I-7

Current and Future Use of Insect Growth Regulators in Crop Protection. H. KAYSER, M. Arslan-Bir, and M. Angst. Syngenta Crop Protection AG, WRO-1060.4.04, CH-4002 Basel, SWITZERLAND. Email: hartmut.kayser@syngenta.com

The idea of "third generation" insecticides once triggered the chemical synthesis of a large number of compounds with physiological actions reminiscent to those of insect juvenile hormones. Methoprene, one of the first compounds developed, has found wide interest as a research tool although its use is limited to public- and animal health. A later compound, fenoxycarb, has proved useful in crop protection due to its improved residual activity. Pyriproxyfen is the only other compound of this mode of action which has found its way into crop protection. This presentation will focus on fenoxycarb. Fenoxycarb has a perfect fit in IPM systems and is used, under the trade name Insegar, for the control of Tortricids, Psylla and scales in fruits. The chemical relationship of the three compounds with juvenile growth factors will be described to provide a basis to the understanding of their mode of action and consequences for their application. As a consequence of their mode of action, fenoxycarb and related compounds express their controlling effect only when applied during either embryonic development or at the late larval stage before pupation. The ovicidal activity is preferred, since the control of the last larval instars does not prevent crop damage. Overall, the narrow biological spectrum limits the use of such "third generation" insecticides to a few selected markets. The resulting small sales potential does not support today's high development costs for newer products.

P-1

Assessing the Persistence of DNA from Leaves of Genetically Modified Poplar Trees. ARMAND SEGUIN, Irene Hay, and Marie-Josée Morency. Natural Resources Canada, Canadian Forest Service, P.O. Box 3800, Sainte-Foy, Quebec, G1V 4C7, CANADA. Email: seguin@cfl.forestry.ca

DNA transformation of forest tree species is now a striking reality and offers the possibility to generate transgenic trees with useful new characteristics. However, it is important to make the proper environmental assessment of these transgenic trees when established in field trials. For instance, the DNA released into the soil by decaying leaves and roots from the transgenic trees may become available for incorporation by soil microbes. The objectives of this study were to establish methods for investigating the persistence of recombinant plant marker genes in degrading transgenic poplar leaf material. We studied the stability of the DNA encoding the neomycin phosphotransferase II resistance marker used in tree genetic engineering. DNA persistence in the environment was determined by placing transgenic poplar leaves in permeable bags that were located on weeds, on the soil and below the soil, and left under natural conditions on the site of a field trial for up to 12 months. This work is the first quantitative analysis of tree DNA stability in a natural forest environment and indicates that fragments of the genetically modified DNA did not remain in the field for more than a few months.

P-2

Transgene Dispersal and Control of Flowering in Poplars S.H. STRAUSS, J. Skinner, A. Brunner, R. Meilan, S. DiFazio, and S. Leonardi. Department of Forest Science, Oregon State University Corvallis, OR 97331. E-mail: Steve.Strauss@orst.edu http://www.fsl.orst.edu/tgerc/

Forest trees are virtually undomesticated and their pollen, and sometimes seed, can move long distances after flowering. To reduce concerns over movement of transgenes into the environment in clonally propagated species such as poplar, we are pursuing means for engineering of complete sexual sterility. As a biosafety mechanism, it is critical to have a high degree of reliability in the trait, thus diverse, redundant mechanisms, and transgene stabilization elements, are desirable. We review our studies of gene dispersal in poplar to illustrate why reduction of flowering is important, then discuss results from studies with floral homeotic genes to study ablation (promoter-cytotoxin), suppression, and dominant negative sterility transgenes in transgenic plants.

P-3

Safety Evaluation of Genetically Modified Forestry Products for Global Regulatory Approvals. PATRICIA SANDERS. Colliant, Inc., 893 N. Warson Rd., St. Louis, MO 63141. E-mail: pat@colliantglobal.com

Genetic engineering of plants is a rapidly developing field of research and commercial opportunity. Current and near-term commercial products include plants that are resistant to pests, including insects, viruses, and fungi; seeds with modified oil or amino acid composition; and herbicide tolerance. Plants with other desirable traits, such as stress tolerance, disease tolerance, drought tolerance, modified carbohydrate metabolism and structural element modifications are under development. As these plants and plant products move toward commercial introduction, gaining regulatory approval becomes a critical step in product development. In the United States, genetically modified plants and plant products are regulated by three federal agencies: the United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). The food, feed and environmental data necessary to support global regulatory approvals of genetically modified plants will be presented. Current issues and anticipated concerns and challenges for forestry species will be discussed.

P-4

In Vitro Culture for Habitat Revegetation: Issues & Opportunities. M.E. KANE and N.L. Philman. Environmental Horticulture Department, P.O. Box 110670, University of Florida, Gainesville, FL 32611-0670. E-mail: mkane@mail.ifas.ufl.edu

Federal and State statutes require restoration of ecological function of degraded wetlands and mined lands, or replacement of destroyed habitats (mitigation), through extensive planting and successful establishment of herbaceous and woody species. Plants materials are typically supplied from donor habitats or nursery propagated stock. Over collection has caused significant ecological degradation to donor habitats. Currently, there are many challenges confronting successful habitat restoration/creation. These include maintenance of genetic diversity, plant source problems, low survival of poorly adapted ecotypes and attainment of ecological structure and function. Various applications of *in vitro* plant technology for selection of naturally and induced genetic diversity, propagation, and long-term storage of plants used for habitat restoration has proven useful to overcome some of these problems. The validity of this approach will be briefly demonstrated from laboratory, greenhouse and field establishment studies, using genotypes of the freshwater wetland species *Sagittaria latifolia*, *Pontederia cordata* and *Spartina bakeri* and the coastal dune grass *Uniola paniculata*.

P-5

Exploring Natural and Tissue Culture-induced Plant Genetic Diversity for Salt Marsh Creation. D.M. SELISKAR. Halophyte Biotechnology Center, College of Marine Studies, University of Delaware, Lewes, DE 19958. E-mail: seliskar@udel.edu

Ecotypes from wild populations and somaclonal variants from tissue culture having specific characteristics that can direct the functional development of the marsh can be selected and propagated for planting in newly created salt marshes. Results from a created salt marsh planted in different ecotypes of the dominant marsh grass, *Spartina alterniflora*, collected from natural marshes along the Atlantic coast of the U.S. suggest that the ecotype of the dominant plant drives the functioning of the marsh ecosystem. Canopy and belowground characteristics remained distinct among ecotypes after five years. The edaphic algal community, aerial microbial decomposers, heterotrophic soil community, and numbers of larval fish utilizing the marsh surface were all controlled by plant ecotype. Results from another common garden study demonstrated that tissue culture regenerants of *Distichlis spicata* and *Sporobolus virginicus* exhibited differences in numerous characteristics that are functionally important, including potential detritus production, belowground organic matter production, canopy structure, and decomposition rate. Combinations of characteristics not found in the wild populations were evident in regenerated lines. Ongoing studies are examining the possibility of selecting somaclones from tissue culture with morphological and biochemical characteristics that can block the invasion of a marsh by *Phragmites*, by making the more desirable plants more competitive with the invasive weed. Using this approach it is possible to propagate marsh grasses that optimize wetland values both at the specific site and at the landscape ecology scale.

P-6

Tissue Culture and Wetland Establishment of the Freshwater Monocots Carex, Juncus, Scirpus, and Typha. SUZANNE M.D. ROGERS. Salem International University, Salem, WV 26426-0500. Email: Rogers@salemiu.edu

Our goals were to regenerate cell cultures of freshwater wetland monocots, acclimatize plants to greenhouse conditions and establish and evaluate plants in wetlands. *Typha* (cattail), *Juncus* (rushes), *Scirpus* (bulrushes) and *Carex* (sedges) were selected for focus because they are common, dominant, high biomass wetland-adapted plants, tolerant of chemically diverse ecosystems. The long-term goal is to develop in situ detoxification strategies by genetically transforming these plants with heavy metal tolerance and remediation abilities. Transgenic wetland plants would be a powerful, cost effective tool for remediation of heavy metal polluted sediments and waters. Success with transforming terrestrial plants with genes for heavy metal extraction and detoxification abilities confirms the effectiveness of this approach. To produce transgenic wetland plants, tissue culture, transformation and plant establishment protocols are needed. Tissue culture systems, defined for numerous monocot crop species, can be readily applied to wetland plants, with a few modifications. Issues to be addressed are selection of suitable explant material, shoot and root regeneration pathways and conditions, culture age vs. regenerability, greenhouse acclimatization needs, plant uniformity and requirements for wetland establishment. Regeneration systems for the rapid cloning/multiplication of wetland monocots, for use in habitat restoration and construction, will be presented. This work was supported by the NASA WV Space Grant Consortium WV EPSCoR, the USDA National Research Initiative Award No. 99-35106-8180 and Salem International University.

P-8

Plant-based Vaccines: Expression and Oral Immunogenicity. H.S. MASON. Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853-1801. E-mail: hsm7@cornell.edu

Plants provide economical alternatives to fermentation-based systems for protein expression. Plants have been shown to faithfully produce antigenic proteins for vaccine use, as well as functional antibodies for immunotherapy, including secretory IgA. We have expressed 3 different candidate subunit vaccines in potato and used the tubers for oral delivery in human clinical trials under IND approved by the FDA. The plant-expressed antigens, E. coli LT-B, Norwalk virus capsid protein, and hepatitis B surface antigen, all stimulated systemic immune responses and where examined, mucosal immune responses. The challenges for future development of edible plant vaccines include increased expression, more effective targeting of antigens to the mucosal lymphoid tissues, and appropriate processing and stabilization of plant material.

P-9

Plant Viruses as an Alternative System For Expression of Foreign Sequences. V. YUSIBOV, S. Spitsin, N. Flyesh, T. Mikheeva, D. Deka, R. Kean, D.C. Hooper, and H. Koprowski. Biotechnology Foundation Laboratories at Thomas Jefferson University, Philadelphia, PA 19107. E-mail: vyusibov@hendrix.jci.tju.edu

A new approach to the production and delivery of vaccine antigens is the use of engineered plant virus-based vectors. Chimeric peptide representing rabies virus G (amino acids 253-275) and N (amino acids 404-418) proteins was PCR-amplified and cloned as translational fusion with the alfalfa mosaic virus coat protein. This recombinant coat protein was expressed in transgenic Nicotiana tabacum (N. tabacum) cv. Samsun NN plants using full length infectious RNA3 of alfalfa mosaic virus or in Nicotiana benthamiana and spinach (Spinacia oleracea) plants using tobacco mosaic virus which lack production of native coat protein. Recombinant virus containing chimeric rabies virus epitope was isolated from infected transgenic N. tabacum cv. Samsun NN plants and used for parenteral immunization of mice. Mice immunized with recombinant virus were protected against challenge infection performed 120 days after the last administration of antigen. We also tested the potential of plant-based experimental rabies vaccine for oral immunization. Earlier, we demonstrated that feeding mice raw spinach leaves expressing rabies epitope resulted in the production of serum IgG and IgA. Therefore, we tested the experimental rabies vaccine based on Av/A4-g24 in human volunteers. Consuming raw spinach leaves containing rabies antigen resulted in the induction of rabies virus-specific serum IgG, IgA or neutralizing antibodies.

P-10

Conjugated Linoleic Acid: a Nutraceutical with Immunomodulatory Properties. J. BASSAGANYA-RIERA, R. Hontecillas-Magarzo, and M.J. Wannemuehler. Veterinary Medical Research Institute, Iowa State University, Ames, IA 50011. Email: bassy@iastate.edu

Conjugated linoleic acid (CLA) is a mixture of positional (i.e., 9, 11; 10, 12; or 11, 13) and geometric (i.e., cis or trans) isomers of conjugated octadecadienoic acid. The CLA mixture has been shown to have anticarcinogenic, antiatherosclerotic, and immunomodulatory properties, but, there is little or no evidence as to which of the individual isomers is most important in mediating these health benefits. The mechanism by which dietary CLA influences immune function could potentially involve regulation of arachidonic acid metabolism, modification of membrane fluidity, and/or transcriptional regulation of gene expression by peroxisome proliferator activated receptor-g. However, these explanations have not been completely accepted in terms of defining the mechanism(s) regulating functional activity because of the lack of molecular evidence in vivo. Other polyunsaturated fatty acids (e.g., a-linolenic or eicosapentanoic acid) with nutraceutical properties are immunossupressive. Conversely, dietary CLA expanded CD8+ lymphocytes and thymocytes (i.e., CD8+ and double negative) in vivo, and enhanced proliferation of CD8+ lymphocytes ex vivo. In addition, CLA decreased the negative impact of mucosal inflammation on growth and intestinal health (i.e., ameliorated intestinal damage associated with mucosal inflammation). Thus, dietary CLA supplementation may facilitate immunological tolerance against nonpathological antigens (i.e., dietary components) while maintaining, or even enhancing, immune reactivity against pathogens.

P-11

Scientific, Regulatory, and Communication Issues in Global Perspective. ROBIN WOO. Georgetown Center for Food and Nutrition Policy, 3240 Prospect Street, NW, Washington, DC 20007. Email: rwoo@iica.ac.cr

Modern agricultural biotechnology is truly an American enterprise—from its academic beginnings a mere 25 years ago, through corporate R&D, to the first comprehensive regulatory regime and retail market introduction. The facile movement of intellectual property from academe to industry has been fostered by a system that provides public funds for basic scientific discovery and a Constitutional tradition of patent protection. The introduction of novel technology has been embraced by a culture that is inherently open to change and improvement in the quality of life. Acres of novel crops have been cultivated on our fertile expanses by technologically sophisticated farmers who maximize food production to nourish a constantly growing global appetite. Therein lies the rub—the global food market is exquisitely sensitive to sociocultural preferences that were not considered in the development of agricultural biotechnology. The first products in the public marketplace did not attract popular demand, and in fact generated some anxiety. Anti-biotech efforts of the European "Green" movement transcended continental boundaries to encourage rejection of this new technology. The global debate has resulted in the development of a variety of regulatory regimes to engender public confidence that approved products are reasonably safe for both public and environmental health and to provide a means for personal choice. This talk will briefly discuss the genesis and impact of this global regulatory movement, in particular focusing on European regulatory efforts and others that are following suit to mandate labeling, as well as the Cartagena Biosafety Protocol. Then we shall take a look at how this all affects the developing world, with a snapshot view of what is happening in Latin America where I am currently on sabbatical.

P-12

Federal Coordinated Framework for the Regulation of Biotechnology in the United States. D.S. HERON. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Riverdale, MD 20737. E-mail: david.s.heron@usda.gov

Over the past two decades, agricultural biotechnology has moved at a rapid pace in the United States and elsewhere in the world. In the early years of the technology, the United States recognized the need to create an overall structure to ensure the safe development of the products of biotechnology. While the decade of the 1970s was one of laboratory experimentation only, the 1980s brought the recognition that genetically engineered organisms were going to require testing and then use in the open environment. In 1986, the "Coordinated Framework for the Regulation of Biotechnology" described the federal agencies which would be primarily responsible for regulating biotechnology in the United States, namely the US Department of Agriculture (USDA), Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). Under this regulatory framework, products are regulated according to their intended use, with some products being regulated under more than one agency. This presentation will address the regulatory framework, philosophy that has guided our efforts, and the progress we have observed. Further information can be found at the interagency web page http://www.aphis.usda.gov/biotech/OECD/usregs.htm.

P-13

"Scientist Communicator" Shouldn't be an Oxymoron: Understanding Our Role in the Food and Agricultural Biotechnology Dialog. C.L. RICHARD. Council for Agricultural Science and Technology, Washington, DC 20002. Email: crichard@st-science.org

If we want to help the public dialog about the safety and potential benefits of biotechnology to be an informed discussion, scientists involved in food and agricultural biotechnology need to share our unique experience with the public. Language and style of communication have been a barrier to our effective communication with an non-scientist public in the past. Yet, we can communicate effectively with the public on these technically difficult topics. Understanding that it only takes a moment to raise doubt and fear in an already emotionally charged and contentious environment, we need to learn how to adapt complex information into brief, lay-language comments. As we explore new ways of communicating with the public, we also need to maintain our credibility and objectivity and the perception that we are credible and objective. We need to communicate early and often in places where the general public looks for information. If we do not Nature abhors a vacuum if not us then someone less objective, less knowledge, with an agenda will fill the void. Public perception will determine the future of biotechnology and other options available to food production and agriculture. We can help provide information so the public can make informed choices.

P-14

The National Research Council Committee on Agriculture Biotechnology, Health, and the Environment. B.A. SCHAAL. Department of Biology, Washington University, St. Louis, MO 63130. E-mail: schaal@biology.wustl.edu

The National Research Council, Board on Biology has convened a Committee on Agricultural Biotechnology, Health, and the Environment. The role of this committee is to maintain surveillance of scientific issues in the areas of plant, animal, and microorganism biotechnology used in food and fiber production and in other emerging applications. The committee considers the use of biotechnology in agriculture and its relationship to health and the environment. Included are diverse applications of biotechnology in areas such as bioremediation, as well as current and future public policy issues. The committee has convened a Workshop on Ecological Monitoring and a subcommittee on the Environmental Impacts of Transgenic Crops. Other projects are underway.

P-15

Consumer Perspectives on Food Biotechnology. CHERYL TONER. International Food Information Council, 1100 Connecticut Ave., Suite 430, Washington, DC 20036. Email: toner@ific.org

International Food Information Council's (IFIC) fifth survey on U.S. consumer attitudes toward food biotechnology indicates consumers are paying attention to the biotechnology issue - or are they? The new survey, conducted January 19-21, 2001 by Wirthlin Worldwide, includes a few new questions to determine how consumers consider food biotechnology in context with other food safety issues. Fall 2000 media coverage focused on the recall of products containing biotech corn not yet approved for food use and the resulting discussions of regulatory decisions. How did this media coverage of a corn product recall affect consumer knowledge and attitudes? More consumers correctly identify corn products as foods currently in the supermarket that have been produced using biotechnology, although overall awareness of the presence of biotech foods in grocery stores has actually decreased since May 2000. Only 1 in 4 consumers had heard anything about recalls of foods produced through biotechnology. When StarLink is named, awareness increases to almost half of consumers, yet 95% state that they have not taken any action in the last few months based on concerns regarding biotech foods. Consumers may have mixed feelings on the labeling issue. When asked, unaided, to identify what information is currently not on food labels that they would like to see added, 74% say "nothing" and only 2% mention "genetically altered." Furthermore, when the current labeling policy is presented to consumers, 70% remain supportive of the Food and Drug Administration (FDA) policy. The survey also presented consumers with the critics' desire to label all foods produced through biotechnology even if the safety and nutritional content are unchanged. When given the critics' view, more than half of consumers agree with them and just over one-third maintain the FDA position. This question represents the most significant shift in the survey perhaps the result of the StarLink episode. However, when consumers were presented with information resource alternatives to the food label in the next question, 75% affirm that information should be provided through toll-free numbers, brochures and web sites "instead of labeling." Consumers continue to respond positively to the benefits of biotechnology for the foods they eat. More consumers are likely to buy foods enhanced to taste better or fresher (58% versus 54% last year), to contain less saturated fat (46% versus 40%, with 33% stating that this benefit would have no effect on their purchasing decision). And consumer acceptance of foods enhanced to require fewer pesticides had remained stable at 70%. For the first time since IFIC began its surveys, the number of Americans expecting to benefit from biotechnology in the future increased. Sixty-four percent expect to benefit from biotechnology in the next 5 years. This finding is consistent with a newly released FDA focus group report that also found consumers "remained open-minded and open to future experience with foods produced by biotechnology." While 79% of those in 1997 expected to benefit, the trend declined to a low of 59% in May 2000 but now appears to be turning upward.

P-16

Using Apomixis in Crop Breeding and Genetics. W.W. HANNA and P. Ozias-Akins. USDA-ARS, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793. E-mail: whanna@tifton.cpes.peachnet.edu

Apomixis is a method of reproduction whereby a chromosomally unreduced egg cell develops into an embryo without fertilization by a sperm. It is common in many polyploid species of plants. Apomixis can be found in wild relatives of agronomic crops such as wheat, maize and pearl millet. Apomixis provides a unique opportunity to maximize crop production around the world. It would allow "fixation" of superior heterozygous genotypes to produce true-breeding hybrids. It would greatly simplify production of commercial hybrids by eliminating the need to produce and maintain inbreds. The need for isolation to increase inbred parents and produce commercial hybrids would also be eliminated. It allows the production, maintenance, and use of unique genotypes. Apomixis is used to produce cultivars in some grasses such as buffelgrass and bluegrass. However, the greatest impact would be to use apomixis to fix hybrid vigor in important world crops such as maize, wheat and rice. Methods for transferring apomixis into cultivated crops include backcrossing (BC) from wild to cultivated species, inducing mutations for apomixis and transformation using molecular techniques. We have worked with all three approaches. The BC approach has effectively progressed to the BC7 generation in pearl millet with the production of apomictic pearl millet-like plants. A major obstacle to overcome with these advanced apomictic plants is poor seed set. Mutations have been identified that appear to be associated with apomixis but none have been advanced to practical application. We have had a major molecular program for a number of years to characterize, map, and clone the apomixis gene(s) so that it could be introduced into major crops via transformation. Lack of genetic recombination has hampered efforts to clone the gene(s). The rapid increase in number of researchers working on apomixis in recent years should enhance the successful harnessing of this important reproductive mechanism.

P-17

Haploid Methods in Wheat and Their Application in Western Canada. J. THOMAS, R. Knox, T. Aung, R. Graf, R. Sadasivaiah, B. Orshinsky, and G. Hughes. Cereal Research Centre, 195 Dafoe Road, Winnipeg, MB, R3T 2M9, CANADA. Email: jthomas@em.agr.ca

For an inbred species like wheat, doubled haploids are needed to accelerate the development of new cultivars and to simplify the genetic analysis of economic traits. A satisfactory haploid method should be simple, efficient, work well with all genotypes, recover a random array of gametes and do no damage to the genome. In wheat, (*Triticum* spp) methods to recover plants from microspores (anther culture; microspore culture) are genotype-dependent and may require several media which may be only partially defined (e.g. ovule co-culture). Spontaneous doubling is frequent with these methods; also anther culture may induce low vigor (somaclonal effects). For methods based on megaspores, recovery of haploids from cultured ovules is very low. Other megaspore-based methods rely on elimination of the chromosomes of particular pollinators from early divisions of the embryo. Where *Hordeum bulbosum* is the pollinator, this method only works with common wheats from the far east. For Eurasian wheats, two dominant genes (Kr1 and Kr2) exclude related pollinators (like *H. bulbosum*) from entering the micropyle. Unexpectedly Kr1 and Kr2 do not exclude pollen of corn (*Zea mays*) or similar tropical grasses so these achieve high fertilisation (ca. 75%) in most wheats. After post-pollination auxin treatments, corn-pollinated wheat ovules enlarge with up to 40% containing an embryo. These embryos are wheat haploids rather than hybrids (corn chromosomes are gone) and, because there is no endosperm, must be excised onto a culture medium. Embryo frequency is affected by genotype but this interacts with the auxin so that high recovery is more often seen with dicamba than with 2,4D. This is especially true for durum or pasta wheats. For chromosome doubling, colchicine is still the agent of choice although potential substitutes have been identified (caffeine and certain herbicides). Both anther culture and corn pollination have been used to produce doubled haploids (DH) for practical wheat breeding programs in western Canada. Based on this experience, interest in anther culture has now waned while corn pollination is being used routinely. Summing over four large research institutions for the year 2000, inputs of $840,000 Canadian (labour, M&S, overhead and facilities) were used to generate about 34,000 DH for a cost of about $25 per line. So far, four DH cultivars have been released to industry. Based on their improved yield, size of the wheat crop and likely adoption we estimate a two-year genetic acceleration of these cultivars to be worth about $200M. Other important results include the use of DH populations to develop genetic maps and markers for critical economic traits. Some new applications and limitations of doubled haploidy are also discussed.

P-18

Towards the Induction of Apomixis: Manipulating Sexual Reproduction in Flowering Plants. J-Ph. VIELLE-CALZADA, G. Acosta-Argüello, W. Huanca-Mamani, and A. Estrada-Luna. Laboratory of Reproductive Development and Apomixis, CINVESTAV-Irapuato, CP 36500 MÉXICO. Email:vielle@ira.cinvestav.mx

The sexual plant life cycle alternates between a diploid and a haploid phase. In contrast to animals, where the meiotic products differentiate directly into gametes, the spores of plants give rise to multicellular haploid structures that differentiate gametes later in their development. Little is known about the genetic basis and molecular mechanisms regulating female gametogenesis in flowering plants. In many species sexual reproduction is replaced by apomixis, a method of asexual reproduction that circumvents female meiosis and fertilization and culminates in the formation of clonal seeds. Differences between the initiation of sexual and apomictic development may be determined by regulatory genes that act during female meiosis and that control events leading to the formation of unreduced female gametes. To test this hypothesis, we are using transposon-based gene trapping approaches as molecular tools to alter sexual development in Arabidopsis thaliana. Genetic screens have yielded several lines conferring restricted GUS expression during early ovule development, some of which are associated with specific mutations altering female gametogenesis. Sequences of genomic fragments flanking the transposon insertion have homology to genes playing important roles in plant and animal development. Their regulatory sequences driving specific gene expression are used to investigate how meiotically derived cells acquire their identity, and to attempt the induction of apomixis initiation in the ovule of potato and corn. Results from these experiments are improving our basic understanding of reproductive development in plants, and will set the basis for a sustained effort in plant germ line biotechnology, a first step toward a flexible transfer of apomixis into a large variety of crops.

P-19

Rooting of Microcuttings: Theory and Practice. GEERT-JAN DE KLERK. Applied Plant Research, Centre for Plant Tissue Culture Research, PO Box 85, 2160 AB Lisse, THE NETHERLANDS. Email: Geert-Jan.de.Klerk@lbo.agro.nl

For transfer to soil, microcuttings may be rooted ex vitro or in vitro. In vitro rooting is often preferable because the plants perform much better during acclimatization. Considerable progress has been made in the understanding of the rooting process using concepts from animal developmental biology. Rooting of apple microcuttings can be dissected into three phases: dedifferentiation during which certain cells develop the competence to respond to the rhizogenic signal (0 - 24h); induction during which auxin exerts its rhizogenic action (24 - 72 h), and morphological differentiation (after 72h). In the latter phase, the signal is not required anymore and, as a matter of fact, previously promoting concentrations are inhibitory during the 3rd phase. For in-vitro rooting, these findings are important for the choice of the type of auxin, for the molecular research on rooting and for research on the mode of action of compounds that influence rooting. Treatments that enhance rooting include pretreatments that result in elongation of stems, and addition of elicitors that facilitate dedifferentiation. When microcuttings are rooted in vitro, the composition of the headspace of tissue-culture containers exerts a major influence on the performance after transfer ex vitro. First, the high humidity results in mal-functioning stomata. Because of this, it is essential that microcuttings generate a well-performing root system as soon as possible after transfer ex vitro. Second, in the headspace ethylene accumulates which may have a very strong detrimental effect on the ex vitro performance after transfer. The effect of ethylene was successfully neutralized by presence of grains coated with $KmnO_4$.

P-21

Influence of Stage II Cytokinin Selection on Rooting and Acclimatization of Native Coastal and Wetland Plants. M.E. KANE and N.L. Philman. Environmental Horticulture Department, P.O. Box 110670, University of Florida, Gainesville, FL 32611-0670. E-mail: mkane@mail.ifas.ufl.edu

Plant growth phase and genotype as well as cultural factors such as mineral nutrition and cytokinin type and concentration can significantly impact successful microcutting rooting and acclimatization. For some species, loss of photosynthetic competence *in vitro* and resultant depletion of carbohydrate reserves ex vitro can negatively impact rooting and acclimatization success. Unfortunately, these interactions are not well documented. Research with native herbaceous wetland and coastal dune species indicate significant differences in cultural requirements for rooting and acclimatization, specifically in benzyladenine (BA) supplemented shoot multiplication media. In the freshwater wetland plant, *Pontederia cordata*, BA, while being the most effective cytokinin for Stage II multiplication, resulted in adverse carry-over effects on ex vitro microcutting survival, rooting and growth compared to when either 2iP or kinetin were used. These carry-over effects were also observed in the wetland species *Sagittaria latifolia* where repeated subculture in greater than 2.5 μ M BA resulted in increased mortality, reduction in multiplication rate, and production ofdormant corms. Evaluation of 28 in vitro propagated genotypes of *Uniola paniculata*, a dune grass species, similarly indicated optimal shoot multiplication in BA supplemented medium but poor (< 1.0%) ex vitro microcutting survival and rooting. Rooting microcuttings for 8 versus 4 weeks in vitro significantly increased ex vitro survival and establishment. This response was attributed to production of photosynthetic competent leaves. BA inhibition of rooting and/or acclimatization may be attributed to an accumulation of stable but toxic BA derivatives such as N^6-benzyladenine-9-glucosyl) benzyladenine-9-riboside [9G]BA. Use of cytokinin-like compounds such as *meta*-topolin [N^6-(-3 -hydroxybenzyl)adenine], that produce rapidly metabolized derivatives, may prove more effective for producing BA sensitive species in vitro.

P-22

Chemically Regulated Zinc Finger Transcription Factors. ULRICH SCHOPFER#, Roger R. Beerli, Birgit Dreier, and Carlos F. Barbas, III. The Scripps Research Institute, La Jolla, CA 92037; #Present address: Novartis Pharma AG, CH-4002 Basel, SWITZERLAND. E-mail: ulrich.schopfer@pharma.novartis.com

Ligand-dependent transcriptional regulators were generated by fusion of designed Cys2-His2 zinc finger proteins and steroid hormone receptor ligand binding domains. To produce novel DNA-binding domains, three finger proteins binding specific 9-bp sequences were constructed from modular building blocks. Fusion of these zinc finger proteins to a transcriptional activation domain and to modified ligand-binding domains derived from either the estrogen or progesterone receptors yielded potent ligand-dependent transcriptional regulators. Together with optimized minimal promoters, these regulators provide 4-hydroxytamoxifen- or RU486-inducible expression systems with induction ratios of up to three orders of magnitude. These inducible expression systems are functionally independent and each can be selectively switched on within the same cell. The potential use of zinc finger-steroid receptor fusion proteins for the regulation of natural promoters was also explored. A gene-specific six finger protein binding an 18-bp target sequence was converted into a ligand-dependent regulator, by fusion with either two estrogen receptor ligand binding domains, or one ecdysone receptor and one retinoid X receptor ligand binding domain. These single-chain receptor proteins undergo intramolecular, rather than intermolecular dimerization and are functional as monomers. Thus, the ability to engineer DNA-binding specificities of zinc finger proteins enables the construction of ligand-dependent transcriptional regulators with potential for the regulation of virtually any desired artificial or natural promoter. It is anticipated that the novel chemically regulated gene switches described herein will find many applications in applied and basic research where the specific modulation of gene expression can be exploited.

P-23

The XVE Inducible Expression System and its Applications in Plant Biotechnology. JIANRU ZUO, Qi-Wen Niu, and Nam-Hai Chua. Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021. E-mail: chua@rockvax.rockefeller.edu

In both basic plant biology research and biotechnological applications, inducible promoters offer numerous advantages and potentials over a constitutive promoter. We have developed an estrogen receptor-based chemical-inducible expression system for use in transgenic plants. A chimeric transcription activator XVE was assembled by fusing the DNA binding domain of the bacterial repressor LexA (X), the transactivation domain of VP16 (V) and the regulatory region of the human estrogen receptor (E). *XVE* expression was controlled by the strong constitutive promoter G10-90. The target promoter consists of eight copies of the LexA operator fused upstream of the -46 35S minimal promoter. The transactivation activity of XVE is strictly regulated by estradiol, a mammalian hormone with no apparent physiological effects on plants. In transgenic *Arabidopsis* plants, estradiol-activated XVE can stimulate expression of a *GFP* reporter gene 8-fold over that transcribed from a 35S promoter without any detectable background expression as monitored by Northern blot analysis. Neither toxic nor adverse physiological effects of the XVE system have been observed in transgenic *Arabidopsis* plants under all tested conditions. The XVE system thus appears to be a reliable and efficient chemical-inducible system for regulating transgene expression in plants. The XVE system has been successfully used in a variety of applications, including overexpression studies, activation tagging as well as the development of a site-specific DNA excision system to remove antibiotic selectable markers from transgenic plants. The chemical-regulated, site specific DNA excision system is a novel combination of the Cre/*lox* DNA recombination and the XVE systems. We placed the *cre* recombinase gene under the control of the XVE system, and two *loxP* sites flanking a DNA segment containing most components of the system, including the selectable marker, XVE and Cre. Upon induction by β-estradiol, DNA sequence sandwiched between the two *loxP* sites was excised from the *Arabidopsis* genome, leading to activation of the downstream *GFP* (green fluorescent protein) reporter gene. Genetic and molecular analyses indicated that the system is tightly controlled, showing high-efficiency inducible DNA excision in all 19 transgenic events tested with either single or multiple T-DNA insertions. The system provides a highly reliable method to generate marker-free transgenic plants after transformation through either organogenesis or somatic embryogenesis.

P-24

A Chemical Gene Switch in *Maize* Using the Insect Ecdysone Receptor. SCOTT VALENTINE. Syngenta, Research Triangle Park, NC 27709. Email: scott.valentine@syngenta.com

A chemical gene switch is a system that regulates gene expression and requires a chemical for induction. In plants, an inducible system will enable the temporal and spatial control of trait expression. This enabling technology will overcome the barriers associated with certain traits, such as sterility and phytotoxicity. Such an inducible system was developed utilizing transcriptional regulation as the mechanism for inducible control of gene expression. Specifically, the nuclear hormone receptor ecdysone (EcR) was employed. The EcR is an inactive transcription factor until binding of an agonistic ligand results in an alteration to an active conformation. Two different EcR systems are currently being studied. One utilizes the *Drosophila* EcR/USP heterodimer and the other uses a lepidopteran EcR alone without USP. Both systems are capable of inducing a reporter gene by using the ecdysteroid tebufenozide as ligand. Data on whole plants will be presented demonstrating a functional gene switch *in planta*.

P-25

The alc Gene Switch: Towards Use in the Field. Alberto Martinez[1], Jackie Paine[1], Richard Dale[1], Mark Caddick[2], Brian Tomsett[2], Ian Jepson[1], and Andy Greenland[1]. [1]Syngenta, Jealotts's Hill International Research Station, Bracknell, Berk, RG12 6EY, UK; and [2]University of Liverpool, Department of Biochemistry, Liverpool, UK. Email: alberto.martinez@syngenta.com

The alc gene switch is derived from the *alc* operon of Aspergillus *niddulans* and has been adapted for use in plants. The system comprises two components, an effector and a reporter cassette. The effector cassette contains the alcR transcription factor under the control of a constitutive promoter while the reporter cassette contains the alcA promoter (alcohol dehydrogenase gene) fused to a reporter gene, *B*-glucuronidase (GUS). The AlcR protein activates gene expression through the alcA promoter in a chemical dependent manner. The alc inducible gene expression system has been demonstrated to activate gene expression in plants after the application of ethanol. Data characterizing the alc gene switch in plants and use in the field will be discussed.

P-26

In Planta Transformation and Insertional Mutagenesis of *Medicago truncatula*. MARIA J. HARRISON. The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. E-mail: mjharrison@noble.org

Many of the recent advances in plant biology made in Arabidopsis have been facilitated by the availability of large populations of T-DNA tagged lines. These populations have contributed to traditional forward genetic approaches by easing the burden of positional cloning and have revolutionized reverse genetic approaches by permitting the systematic identification of 'knock out' mutants in known genes. A range of T-DNA's have been utilized in large-scale insertional mutagenesis programs in Arabidopsis. One particularly versatile version is the activation-tagging T-DNA, which carries 4 copies of the CAMV 35S enhancers adjacent to the right border. Insertion of this T-DNA into the genome results not only in loss-of-function mutants, but also dominant gain-of-function mutants due to the activation of adjacent genes by the 35S enhancers (Weigel et al., 2000). *Medicago truncatul*a is a legume that is widely used as a model for the analysis of the nodulation and arbuscular mycorrhizal symbioses, as well as other unique aspects of legume biology. We have initiated insertional mutagenesis of *M. truncatula* using the SK015 activation-tagging vector (Weigel et al., 2000) and an *in planta* transformation method developed for *M. truncatula*. Based on a median gene length of 2.1 kb (Bevan et al., 1998) and a genome size of 450 Mb, we estimate that 260,000 random insertions throughout the *M. truncatula* genome are required to achieve a 90% chance of obtaining an insertion in a given gene. *In planta* transformation of *M. truncatula* and progress towards a creation of a large activation-tagged population will be discussed.

P-27

Gene Function Discovery with Plant Viral Vectors. G. DELLA-CIOPPA. Large Scale Biology Corporation, Vacaville, CA 95688. E-mail: guy.della-cioppa@lsbc.com

A slow, labor-intensive process requiring the production of hundreds to thousands of individual transformation events has traditionally bottlenecked the discovery of new genes conferring useful phenotypes in plants. GENEWARE viral vector technology developed at Large Scale Biology Corporation represents new technology for achieving rapid, high-level expression of genes in plants. We have developed vectors based on plus-sense RNA viruses that can be packaged in the laboratory and used for large-scale transfection of the preferred host organism. Genomics applications of the technology involve high-throughput determination of unknown gene function based on sense and/or antisense production in the cytoplasm of uncharacterized RNA species.

P-28

RescueMu, a Novel Mutagenesis and Gene Recovery Tool in Transgenic Maize and Wheat. MANISH N. RAIZADA. Laboratory of Crop Genomics, Department of Plant Agriculture , University of Guelph, Guelph, ON CANADA N1G 2W1. E-mail: raizada@uoguelph.ca

The *MuDR/Mu (Mutator)* transposons of maize represent a powerful tool for insertional mutagenesis to define gene function (functional genomics). *MuDR/Mu* transposons have several unique features for this purpose: (1) they can duplicate to high copy numbers, a useful feature when mutagenizing organisms with large genomes; (2) they insert randomly to both linked and unlinked chromosomal sites and thus are useful for random mutagenesis experiments; (3) they have a very strong preference for inserting into genes not repetitive DNA, thus effectively reducing the size of the target genome; and finally (4) *MuDR/Mu* transposons primarily insert late in the germline, thus creating independent insertion events among sibling progeny. For these reasons, *MuDR/Mu* transposons are the most widely used tool for gene discovery and allele definition in maize. To facilitate quick gene recovery following a *Mu* insertion, we have created transgenic maize plants with a *Mu1* element harboring a bacterial plasmid. We have called this new element *RescueMu*. By crossing these lines to an active transposase source (encoded by *MuDR*, and performing plasmid rescue, we can now go from mutant maize plant to isolated gene in less than 10 days, despite the large size of the maize genome. These *RescueMu* lines are now being used to develop a large collection of germline gene knockouts in maize, ammenable to plasmid rescue and DNA sequencing. I will also describe our efforts to transfer *Mutator* and *RescueMu* transposition to hexaploid and diploid wheat. The maize project is in collaboration with Dr. Virginia Walbot at Stanford University. The wheat project is in collaboration with Dr. Alessandro Pellegrineschi of CIMMYT, and Dr. Mark Jordan and Dr. Linda Harris of Agriculture and Agri-Food Canada.

P-30

Importance, Status, and Limitations of Cassava Transformation. C.M.FAUQUET. ILTAB, Donald Danforth Plant Science Center, Center for Molecular Electronics, Room 308, 8001 Natural Bridge Road, St. Louis, MO 63121. E-mail: ILTAB@DANFORTHCENTER.ORG

Cassava constitutes the third source of calories in developing countries and more than half a billion people eat cassava on our planet. For the last 30 years the production of cassava has significantly increased in the world mostly by increasing the cultivated surfaces. The average increase in productivity was less than 1% per year. Therefore to feed a booming population in developing countries, to improve their diet and to save space, forest and energy, cassava productivity and quality must increase dramatically in the coming 30 years! In order to achieve that goal, genetic transformation is the only viable option to integrate traits that will improve the quantity and quality of cassava cultivars appreciated by the farmers. A few years ago, ILTAB developed a technology to integrate genes into cassava via embryogenic suspensions and since that time, more than 125 transgenic lines have been produced. We also demonstrated that it is possible to produce similar suspensions with a variety of cassava cultivars from different parts of the world. The transgenic plants are non-chimeric, are stably expressing the transgenes over time and useful genes for virus resistance and other traits are being integrated into cassava. However we are facing two types of difficulties that have to be surmounted in order to transfer that technology to relevant countries and use it more extensively for many other beneficial traits. The first constraint is the difficulty of transferring transgenic cassava plants to less developed countries and to Africa in particular, in order to perform field trials, due to the lack of biosafety regulations in most of the countries where such field trials could be performed. The second constraint is the difficulty of transferring this technology to a large number of cassava cultivars in the world in order to be used by the farmers. To alleviate this second constraint, capacity building at different levels in cassava producing countries, including tissue culture training, will be indispensable in the coming years. To solve these problems, ILTAB at the Danforth Center and CIAT in Colombia are developing a Global Plan of Action to make better use of these new technologies.

P-31

Genetic Transformation of Some Tropical Species. Miguel A. Gómez Lim. CINVESTAV-Irapuato, Department of Plant Genetic Engineering, Km. 9.6 Carretera Irapuato-Leon, Apartado Postal 629, Irapuato, GTO 36500, MEXICO. Email: mgomez@ira.cinvestav.mx

Biotechnology has had a dramatic impact in different fields. This is particularly evident in horticulture where the application of biotechnology (genetic engineering) has produced, in a short period of time, exciting results. Clearly, an essential prerequisite has been the development of methods for in vitro culture and regeneration of the crop. In this respect, tropical trees have proved somewhat difficult to in vitro manipulation. Nevertheless, it is now possible to genetically manipulate several tropical crops with varying efficiencies. This has opened the door for a number of interesting possibilities to control a series of diseases and to extend the postharvest life, a problem that particularly afflicts some tropical fruits. During the talk, the progress on the genetic transformation of three very important tropical crops, mango, avocado, and banana, will be reviewed, and ongoing work at CINVESTAV-Irapuato and other places, aimed at modifying specific traits will be discussed.

T-1

A Reduction of DNA Repair Capacity by Endocrine Disrupters in Testicular Cells. JAMES W. DUMOND, JR. Department of Environmental Health Sciences, School of Public Health, University of Alabama at Birmingham, Birmingham, AL 35294. Email: JDumond@ms.soph.uab.edu

In order to gain a better understanding of how endocrine disrupters might act as initiators of tumor development, we have developed a molecular model. The focal point of this model is the non-genotoxic induction of genomic instability, as we have hypothesized that mitogens can act as initiators through a perturbation of the cell cycle. Specifically, we believe that a reduction of cell cycle time will reduce the cell's overall DNA repair capacity. We have assayed diethylstilbestrol and Bis-phenol A for its ability to reduce DNA repair activity in the testicular Leydig cell line TM3, using a modified host cell reactivation assay. Briefly this *in vitro* assay uses plasmid DNA, with a reporter gene within the DNA sequence. When transfected in to a host cell, the reporter gene, (in our studies CAT), will be expressed. The level of expression is then determined at various time points using a standard CAT assay. The same methodology is used to test the DNA repair capacity of the host cells, except the plasmid DNA is damaged so that it cannot express a functional gene product. Thus expression of a functional gene product is the result of DNA repair. The results of this preliminary data are supportive of our overall model in that a reduction of repair was noted with a decrease in cell cycle time.

T-2

In Vitro Assessment of Endocrine Disruptors: Activity of the Environmental Estrogen Bisphenol A at Levels of Current Human Exposure. WADE V. WELSHONS[1], Susan C. Nagel[2], Barbara M. Judy[1], Julia A Taylor[1], Kembra L. Howdeshell[2], Rachel L. Ruhlen[2], and Frederick S. vom Saal[2]. [1]Dept. Veterinary Biomedical Sciences and [2]Div. of Veterinary Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211. Email: WelshonsW@missouri.edu

We developed approaches in vitro to predict the endocrine disruptor bioactivity of compounds in the near-physiological range of hormonal activity in animals and humans. To evaluate estrogenic activity and effective free concentration determined by how the compound is carried in serum, we developed a relative binding affinity-serum modified access (RBA-SMA) assay to determine the access of xenobiotic estrogens from 100% serum to estrogen receptors within intact MCF-7 human breast cancer cells. The RBA-SMA assay indicated that many compounds, including the ubiquitous plastic monomer bisphenol A (BPA) and the positive control diethylstilbestrol (DES), showed enhanced estrogenic activity (relative to estradiol) when reaching cells from serum, than when tested in serum-free conditions. The results predicted endocrine disrupting effects of BPA at very low exposures to the fetus, within the range of current human exposures to this chemical, and this was confirmed in the offspring of pregnant mice exposed to BPA at oral doses down to 2 μg/kg/day. Effects of the maternal exposure on the offspring included reduced sperm production and enlarged prostate gland with up-regulated androgen receptors in males, earlier puberty and changed uterine responsiveness in females, and increased body weight at weaning in both males and females. These effects were brought about by fetal exposure to several estrogens, implying an estrogenic mechanism for all of the effects. To acquire information that can be used to develop in vitro pharmacokinetic models, we have also studied the distribution in intact animals from oral exposure to circulating concentration by use of tritium-labeled BPA (high specific activity). Our results indicated that BPA bioaccumulates in pregnant but not in nonpregnant female mice, increasing exposure at a critical developmental time, and further indicating multiple mechanisms by which BPA can be highly active as an endocrine disruptor in the fetus. Supported by University of Missouri VMFC0018, NIH CA50354 and NIH ES08293.

T-3

Use of an In Situ Ovarian Cell System to Study Effects of Phyto- and Synthetic Estrogens on Apoptosis. TODD A. WINTERS, Nicole A. Hoefling, Angela M. Raymer, and William J. Banz. Departments of Animal Science, Food and Nutrition, and Physiology, Southern Illinois University, Carbondale, IL 62901. Email: tw3a@siu.edu

Phytoestrogens and other environmental estrogens have been reported to modulate reproduction in exposed animals and humans. Our research group has been studying the effects of estrogenic endocrine modulators on the ovary. The ovary is known to be responsive to estrogens primarily through estrogen receptor-â. Specifically, the objectives of these studies have been to elucidate how soy isoflavones and other estrogenic compounds affect granulosa cell apoptosis (programmed cell death), the underlying mechanism for follicular atresia. Porcine granulosa cells are collected by fine-needle aspiration and plated on 8-chamber poly-L-lysine coated microscope slides at 750,000 cells/well. The slides are incubated at 37°C/5% CO_2 for 24 h, then treated for another 24 h with estrogenic compounds, antiestrogens and/or other endocrine modulators (0.1 n*M* to 10 ì*M*). The cells are then processed using a terminal deoxytransferase-mediated dUTP digoxigenin nick end labeling (TUNEL) *in situ* apoptosis assay (Intergen, Purchase, NY). The apoptotic cells stain brown with diaminobenzidine (DAB) stain, while viable cells stain blue-green with methyl green counterstain. The percentage of apoptotic vs. non-apoptotic cells are quantified using an image analysis system (Optimas, Edmonds, WA). To date we have found that genistein, daidzein, glycitein, (-)-bisdehydrodoisynolic acid, diethylstilbesterol, and estradiol-17â inhibit apoptosis in a dose-responsive manner; therefore potentially increasing ovulation propensity of the porcine ovary. This effect is eliminated by co-treatment with the antiestrogens 4-hydroxytamoxifen or ICI 182,780, strongly suggesting that the anti-apoptosis effect is estrogen receptor-modulated. Using this system, we are also investigating how these environmental estrogens interact with other classical modulators of ovarian function. We believe this *in vitro* system could further be used to study other potential endocrine modulators of reproduction.

T-4

Detection of Environmental and Occupational Estrogenic Chemicals- Induced Mutations in Mouse Leydig Cells by RAPD/ AP-PCR Fingerprinting. KAMALESHWAR P. SINGH, James W. DuMond, and Deodutta Roy. Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL 35294. Email: royd@uab.edu

Many environmental and occupational estrogenic compounds are known to produce adverse effects, including cell transformation, apoptosis and tumorogenesis. The molecular mechanisms of these actions of estrogenic chemicals are not clear. Recently, select estrogenic chemicals, 17 beta-estradiol (E2), 2-methoxyestradiol (2-MeOE2), 2-methoxyestrone(2-MeOE1), 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol(4-OHE2), have been shown to induce aneuploidy and mutations in *HPRT* gene in Syrian hamster embryo(SHE) and Chinese hamsterV79 cell culture model. Now it is widely accepted that phenolic estrogens are genotoxic. It is important to understand the sequential genetic events involved the process of conversion of normal cells into tumor cells by estrogenic compounds. Many methods are available to assess DNA damage and mutations. Most of them are designed to detect genetic changes in the known gene(s)/region(s) of the genome. Though, the gene chip/micro array technology is very useful for the detection of genome-wide changes in gene expression including the mutations-associated altered gene expressions, however, for localization of mutations in genes showing increased or decreased expression, post array analyses have to be carried out. Random amplified polymorphic DNA (RAPD), also known as arbitrary primed polymerase chain reaction (AP-PCR), is a method for the detection of mutations at the genome-wide level. RAPD/AP-PCR has been extensively used to detect genetic polymorphism and genetic variation in the plants, microbes and to a limited extent in the human and experimental animals, however, AP-PCR potential has not been fully utilized for the screening of mutagenic potential of chemicals. In this study, we have examined the mutagenic activity of estrogenic chemicals, diethylstilbestrol (DES), 17 α and 17 β estradiol, bisphenol A, α-zearalanol. Leydig cells (TM3) were exposed to different concentrations of each compound. Genomic DNA was amplified using ten-mar random primers, and the amplification products were resolved on agarose or sequencing gels. Comparison of RAPD fingerprints of treated and untreated DNA revealed homozygous deletions and insertions of amplified products in TM3 cells treated with 100 ng/ml concentrations of DES and Zearalanol for 72 hrs. Our study for the first time show that RAPD/ AP-PCR is very useful for the detection of environmental and occupational estrogenic chemical-induced mutations.

T-5

Transporter Localization and Drug Disposition in Multidrug Resistant Cancer Cells. D.C. WILLIAMS. Lilly Research Laboratories, Indianapolis, IN 46285. Email: danw@lilly.com

The expression of certain members of the ABC superfamily of transporter proteins is associated with multidrug resistance in cancer cells. Data has accumulated implicating MDR-1 (P-glycoprotein) and members of the MRP (multidrug resistance-associated protein) family in clinically relevant drug resistance. Transporter protein function is dependent both on the level of expression of the proteins and on their topographical localization within cells. Expression and localization of transporters in cells is dependent on the cell's history, culture condition and degree of differentiation. We use fluorescence immunocytochemistry with confocal microscopy and flow cytometry to characterize the expression and localization of transporters in cultured cells induced to over-express transporters by chronic exposure to drug and cells transfected with transporter genes. When possible, we use paired cell lines in which a non-induced or vector control cell line is compared to a derived/transfected cell line expressing the transporter protein. The expression of transporters in cells is reflected in changes in drug distribution in the cells following short-term exposure to drugs. We have compared drug distribution in paired cell lines by flow cytometry and confocal microscopy using naturally fluorescent drugs (e.g., daunorubicin, doxorubicin, mitoxanthrone), drugs conjugated to fluorescent markers (e.g., BODIPY paclitaxel), or fluorescent drug surrogate transporter substrates (e.g., rhodamine 123, calcein AM). Pharmacological modulators of transporter function can be shown to affect drug distribution in cells.

T-6

The MRP Subfamily of Drug Transporters. G.D. KRUH. Medical Sciences Division, Fox Chase Cancer Center, Philadelphia, PA 19111.

The MRP subfamily of ABC transporters from mammals consists of at least seven members, six of which are known to transport amphipathic anions. MRP1, MRP2 and MRP3 bear a close structural resemblance, confer resistance to a variety of natural products as well as methotrexate, and have the facility for transporting glutathione and glucuronate conjugates. MRP1 is a ubiquitously expressed efflux pump for the products of phase II of xenobiotic detoxification, while MRP2, whose hereditary deficiency results in Dubin-Johnson syndrome, functions to extrude organic anions into the bile. MRP3 is distinguished by its capacity to transport the monoanionic bile constituent glycocholate, and may function as a basolateral back-up system for the detoxification of hepatocytes when the usual canalicular route is impaired by cholestatic conditions. MRP4 and MRP5 resemble each other more closely than they resemble MRPs 1-3 and confer resistance to purine and nucleotide analogs which are either inherently anionic, as in the case of the anti-AIDS drug PMEA, or are phosphorylated and converted to anionic amphiphiles in the cell, as in the case of 6-MP. Given its capacity for transporting cyclic nucleotides, MRP5 has also been implicated in a broad range of cellular signaling processes. The drug resistance activity and physiological substrates of MRP6 are unknown. However, its hereditary deficiency results in pseudoxanthoma elasticum, a multisystem disorder affecting skin, eyes and blood vessels. It is hoped that elucidation of the resistance profiles and physiological functions of the different members of the MRP subfamily will provide new insights into the molecular basis of clinical drug resistance and spawn new strategies for combating this phenomenon.

T-7

Drug Uptake and Efflux Transporters: In Vitro to In Vivo Relevance. R.B. KIM. Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232.

Molecular characterization of drug metabolizing enzymes, such as the cytochrome P450 monooxygenases, has led to an improved understanding of the specific function, expression, and genetic heterogeneity of the individual genes and gene products. While metabolism is clearly important in drug disposition, it is becoming evident that drug transporter proteins may have a similar role. However, unlike drug metabolizing enzymes, identification of drug transport systems at the molecular level, for the most part, has only recently been attained. Accordingly, this has meant that the extent of our knowledge, regarding the role of drug transporters in drug disposition and drug-drug interaction is not as extensive. Nevertheless, there is increasing evidence to support an important role of drug transporters in drug disposition and in disease states. During this session, drug uptake and efflux transporters of potential relevance to drug disposition will be systematically reviewed. Emphasis will be given to model cell systems suitable for functional characterization of transporter activity.

T-8

Selection and Use of Appropriate Skin and Epithelial Models for Product Testing. K.M. MARTIN. Johnson & Johnson Consumer Products Worldwide, Skin Research Center, Skillman, NJ 08558. E-mail: kmartin1@cpcus.jnj.com

Differentiated multilayered models of human skin and epithelia allow the assessment of fully formulated products and aqueous incompatible materials and as a result play an important role in preclinical safety assessment. Several models are commercially available, and offer a convenient, safe, reliable and reproducible supply of tissues. Other models are prepared or modified in house. Each model offers various advantages and selecting the most appropriate model will depend on the specific application and area of interest. Important considerations include the presence of target cell populations, epithelial -mesenchymal interactions, and responsiveness. In this context, a comparison of epithelial and full thickness skin models will be discussed and data on melanocyte containing models and models used for predicting vaginal irritation will be presented.

T-9

Evaluation of the Usefulness of 3-D-Models of Reconstituted Human Skin and Epidermis in Applications of Regulatory Skin Toxicology: Prevalidation, Validation, Catch-Up-Validation, and Regulatory Acceptance.

M. LIEBSCH. ZEBET BgVV, National Center for Evaluation and Validation of Alternatives to Animal Experiments, D-12277, Berlin, GERMANY. Email: liebsch.zebet@bgvv.de

In 1991 we evaluated a model of full human skin (Skin^2, ATS, La Jolla) for phototoxicity testing. The test developed showed promising results as an adjunct to a test performed with Balb/c 3T3 cells. While it detected acute phototoxins correctly, it classified those chemicals negative, that are known photoallergic in humans, but are not phototoxic after single application (e.g., musk ambrette, 6-methycoumarin). The test was later evaluated in a blind trial with 30 chemicals with promising outcome. When the production of Skin^2 was terminated in 1996, we evaluated together with P&G (Cincinnati, OH) and Beiersdorf AG (Hamburg, D) if human reconstituted epidermis, EpiDerm, (MatTek, Ashland, USA) could replace the full skin model. The outcome was excellent, confirming reproducibility between labs, and over time, as well as high predictivity. Currently, we are evaluating, if the EpiDerm test can sufficiently be used for photosafety testing of formulations and for testing of phototoxic potency. In a European validation study of four in vitro tests for predicting corrosive potential to the skin, two tests provided good predictivity, and have meanwhile gained full acceptance in the EU for regulatory applications. One of the accepted tests is using EPISKIN, a human reconstituted epidermis model. Since EPISKIN is currently not available, we have performed a 'catch-up-validation study' together with two laboratories, BASF (Ludwigshafen, D) and HLS (Huntingdon, UK) to prove equivalence of EpiDerm with regard to structure an performance criteria. This study has meanwhile led to EU acceptance of the test. Current attempts to evaluate the use of 3D skin/epidermis models for skin irritancy testing and for assessment of percutaneous absorption will be addressed.

V-1

Tissue Engineered Cell Therapy for Skeletal Tissues. ARNOLD I. CAPLAN. Skeletal Research Center, Biology Department, Case Western Reserve University, Cleveland, Ohio 44106.

The assisted regeneration of various human adult tissues is currently not possible even though every tissue has repair/regeneration/turnover capacity. To accomplish regeneration of such tissues, several basic principles must be followed; with the elucidation of these Tissue Engineering Principles, several success in pre-clinical models have been reported. Our laboratory uses the simplifying assumption that successful tissue repair/regeneration must mimic or recapitulate selected aspects of embryonic events. This logic is coupled with the realization that some of the natural factors involved in the response to tissue injury and subsequent natural repair must be inhibited to facilitate assisted regeneration events. Central to all of our experimental efforts is the basic *in vitro* technology of mititoically expanding the desired cell types (s) to provide adequate access to cells with reparative potential and, thus, our use of the term "cell therapy." We have focused on the use of adult marrow-derived Mesenchymal Stem Cells (MSCs) capable of differentiation into one of several skeletal phenotypes. Data will be presented to document: (1) The isolation and expansion of MSCs without loss of their developmental potential. (2) The choice of delivery vehicles to repair several different skeletal tissues with the focus on their support of and involvement in subsequent reparative events. (3) The *in vitro* challenges required to increase the dimensions of implant materials to be useful for humans with clinical need for assisted regeneration; and (4) The difficulties in translating *in vitro* cellular capacities to *in vitro* sites. Supported in part by grants from National Institute of Health.

V-3

Tissue Engineering Bioreactors. G. VUNJAK-NOVAKOVIC. Massachusetts Institute of Technology, Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139. Email: gordana@mit.edu

Tissue engineering can provide functional constructs for controlled an *in vitro* studies of tissue development and in vivo repair. The approach discussed in this talk involves the use of an *in vitro* environment that embodies the biochemical and physical signals that regulate tissue development and maintenance *in vivo*. The key components of such tissue engineering system are: (i) isolated cells (e.g., obtained from cartilage, heart muscle, or bone marrow; embryonic stem cells) that can be expanded, and transfected to express the genes of interest, (ii) polymer scaffolds that provide a defined 3-D structure for tissue development and biodegrade at a controlled rate, and (iii) bioreactors that maintain the conditions necessary for the cells to regenerate functional tissues. Tissue engineering using cell-polymer-bioreactor system will be discussed with respect to various factors that can be utilized to modulate tissue growth and function: cell source (primary or expanded cells; precursor cells; gene transfer), initial cell density, scaffold structure (mesh or sponge), composition and degradation rate, biochemical and physical regulatory signals, mass transfer rates of oxygen, nutrients and metabolites, flow conditions (convective flow vs. direct perfusion; laminar vs. turbulent; steady vs. dynamic) and the duration of bioreactor cultivation. Construct structure and function can be assessed using quantitative methods (e.g., biochemical, histological, and ultrastructural analyses, expression of tissue specific markers, mechanical responses and integrative potential in vitro and in vivo for engineered cartilage, electrophysiological behavior for engineered cardiac tissue). Functional construct properties (e.g., biomechanical parameters, integrative potential, biosynthesis rates of matrix components) can be correlated to the construct structure and the conditions and duration of bioreactor cultivation. The spatial and temporal patterns of tissue development were rationalized using point-to-point mapping of tissue composition in conjunction with mathematical modeling. Most recently, gene transfer of growth factors was utilized to enhance the structure and function of in vitro grown tissues.

V-4

Cationic Lipid Based Gene Transfer. R. K. SCHEULE. Genzyme Corporation, 31 New York Avenue, Framingham, MA 01701. E-mail: ron.scheule@genzyme.com

Of the several different kinds of non-viral vectors under development over the past decade, cationic lipid-based systems have been evaluated in perhaps the greatest detail. Several features of these systems, including their ease of construction and the lack of adaptive immune responses against their components, have made them attractive alternatives to the more potent but immunogenic viral-based gene transfer systems. Although some aspects of cationic lipid based gene delivery systems can be optimized *in vitro*, their ultimate ability to effect gene transfer by a given admnistration route must be evaluated *in vivo*. Through extensive screening of many hundreds of cationic lipid based formulations, optimized gene delivery systems have been developed for several clinical applications, including genetic diseases and cancer. For cystic fibrosis for example, aerosol formulations of cationic lipids have been developed and translated into clinical trials, where they have shown some, albeit low, efficacy together with some minor toxicity. Recurring challenges with these vector systems include: (i) increasing their persistence of expression, and (ii) improving their therapeutic index by increasing the potency of the formulations while decreasing their toxicities. Significant increases in potency have been realized recently by generating formulations from smaller and more homogenous cationic liposomes. The toxicities resulting from these vector systems have been characterized and the DNA component correspondingly reengineered to minimize the innate immune responses that have been found to accompany vector administration. Coupled with alternative promoters, these "second generation" synthetic vector systems have been refined to give higher, more persistent expression with less toxicity than first generation systems. Taken together, these recent improvements in cationic lipid based gene delivery systems represent a significantly improved platform for therapeutic gene delivery.

V-5

Gene Gun Applications: In Vivo Gene Expression Regulated by Tissue-Specific Promoters. MICHAEL T.S. LIN. Department of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, PA 19107. E-mail: Michael.Lin@mail.tju.edu

The gene gun is becoming a powerful tool in the study of the fundamental mechanisms of gene delivery, gene regulation, and gene therapy. Specifically, the effects of epidermal-specific promoters on gene expression can be rapidly and efficiently assessed in in vivo models. The effect of tissue-specific promoters on gene expression was analyzed in murine skin in vivo and in cell culture. The expression b-galactosidase, a reporter gene, was compared among three promoters: involucrin, keratin 14, and cytomegalovirus. The Heliosâ gene gun was used to introduce plasmid DNA to BALB/C mice, in vivo. Skin biopsies were taken for histology and b-galactosidase staining after 24 hours. In tissue culture cells, plasmid DNA was delivered by transient transfection to 293 (transformed primary human embryonal kidney), NIH 3T3 (immortalized mouse fibroblast), and human keratinocytes. b-galactosidase expression was analyzed by histochemical staining and chemiluminescence. With the K14 and INV promoter constructs, b-galactosidase gene expression was detected only in the epidermis. With the CMV promoter, b-galactosidase could be detected in both the dermis and epidermis. In cell culture, the INV and K14 promoter constructs showed significant b-galactosidase expression in human keratinocytes, but little expression in 293 and NIH 3T3 cell types. With the CMV promoter constructs, significant expression could be detected in all cell types. The regulation of gene expression by different promoters can be demonstrated in vivo and in cell culture. In cutaneous gene therapy applications, endogenous promoters may be used to regulate the expression of delivered genes in different cell types.

V-6

Development of Gene Therapy for Hemophilia B: Gene Regulation In Vitro and In Vivo, and Gene Transfer Vector Systems. KOTOKU KURACHI. Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-0618. E-mail: kkurachi@umich.edu

Disorders in blood coagulation cause substantial morbidity and mortality in the US and worldwide. Among these disorders, hemophilia B, deficiency of factor IX in the circulation, is one of the most extensively studied and frequently targeted as a valuable recessive disease model for developing gene therapy methods, using viral, non-viral gene delivery vectors or via in situ gene modifications. Despite many different gene transfer approaches, only marginally positive clinical trial results have been achieved to date, indicating difficulty in overcoming some fundamental obstacles involved in developing a highly effective gene therapy even for such a well defined, plasma protein disorder. An ideal gene therapy method to be developed for this genetic disorder should be safe and long-term effective (at least years), if not for the entire life-span of patients, after a single or a few number of repeated therapies. For developing such a therapy for this disorder, deep understanding of the age-regulatory mechanisms of the natural factor IX gene becomes crucial. Age-dependent regulation of the human factor IX gene involves two critical genetic elements, ASE and AIE, which are needed for age-stable expression and age-dependent expression increase of the gene (Kurachi et al., Science 285:739; 1999). More recently, we successfully demonstrated that these elements universally function with different genes, thus opening exciting possibilities of their application in developing highly refined gene delivery vector systems. ASE element is of particular importance in this aspect, and its utility in different expression vector/promoter contexts, for instance, viral vectors including adeno-associated virus (AAV) and adenovirus, is of great interest and under testing in mice. Age-dependent regulation of factor IX gene expression and its critical implication in developing hemophilia B gene therapy approaches will be presented in a comprehensive perspective.

V-7

Electrically Enhanced Delivery of Plasmid DNA. RICHARD HELLER, Loree Heller, M. Lee Lucas, Richard Gilbert, and Mark J. Jaroszeski. Department of Surgery, Center for Molecular Delivery, College of Engineering, Univ. of South Florida, Tampa, FL 33612. Email: rheller@hsc.usf.edu

The efficient delivery of therapeutic molecules is an important tool for the treatment of a variety of diseases. A common problem of gene therapy treatments is inefficient gene delivery and insufficient expression. Typically, the goal is to target gene delivery to a particular type of cell or to cells within a specific tissue. The delivery of genes that code for biologically active compounds is envisioned as a treatment for many diseases including cancers and metabolic disorders. The uptake of molecules through the cell membrane can be facilitated by use of electroporation, a physical phenomena that temporarily permeabilizes cell membranes. When membranes are in a permeabilized state it is possible for molecules that do not normally pass through the membrane to gain intracellular access. Electroporation has been used in vitro, ex vivo, and in vivo to delivery drugs or plasmid DNA either alone or in combination. With respect to gene transfer, our group has initiated several studies to investigate the use of electroporation for plasmid DNA delivery in a variety of tissues. Protocols were developed to allow delivery of the plasmid directly to either tumor, normal skin, normal liver, or normal muscle. It was determined that different electric pulse conditions were needed to obtain peak expression at each of the various sites. However, in each case expression levels were significantly increased when compared to injection alone. To test the therapeutic potential, several cytokine genes were delivered to tumors either alone or in combination. Although the work is in its initial stages, encouraging results have been obtained. Long term complete regressions have been obtained with various treatment protocols of a murine melanoma. This work is being continued and expanded to optimize the procedure and confirm these initial results. (Supported by research grants from the NIH - R21 DK055588 and the Center for Molecular Delivery, Univ. of South Florida).

W-1

The Challenge of Choosing Controls for Bioreactor Studies of Cells and Tissues. GORDANA VUNJAK-NOVAKOVIC. Massachusetts Institute of Technology, Division of Health Sciences and Technology, 45 Carleton St., MIT E25-342, Cambridge, MA 02139. Email: gordana@mit.edu

Tissue culture bioreactors permit the in vitro cultivation of cells and tissues under the conditions of precisely controlled pH, temperature, chemical and physical regulatory signals. However, any change in hydrodynamic conditions within bioreactors (e.g., flow and mixing patterns) is generally associated with changes in (a) mass transfer rates (e.g., of gases and nutrients) and (b) physical signals (e.g., shear, pressure) acting at the cells. In many cases, it is difficult or even impossible to change only one parameter at a time. The interplay between the physical and chemical signals in the cell microenvironment must thus be well understood in order to select appropriate controls and analyze experimental data. This talk will discuss the selection of controls and data analysis in bioreactor studies of cells and tissues.

W-2

The Importance and Application of the Prediction Model to In Vitro Biology. L.H. BRUNER. Gillette Medical Evaluation Laboratories, Needham, MA 02942. E-mail: leon_bruner@gillette.com

Considerable research has been done in an effort to develop in vitro methods that can be used for predicting toxicity in vivo. The evidence that an in vitro test can adequately predict a toxic response is usually generated early in its development. This evidence is obtained by evaluating a training set of test substances in the new in vitro test and comparing its results with the respective in vivo toxicity of each substance. The data derived from this research are used to construct an algorithm that allows toxicologists to convert the results from the in vitro test into predictions of toxicity in vivo. These algorithms are called prediction models. A prediction model is essential because it defines exactly how to convert the results from an in vitro test into predictions of in vivo toxicity. The ability to make correct predictions is extremely important since this information is what a toxicologist uses to make decisions during a safety assessment. If an in vitro test does not have an adequate prediction model, there is no way to use it. An alternative non-animal test therefore consists of two key components: the in vitro test procedure and its prediction model. Both of these components must be validated before the test is used in the safety assessment process. The presentation will provide an overview of the approaches used to develop prediction models, how to validate them, and how to use them.

W-3

The Need for Controls Focused on an Assay's End Points J.W. HARBELL. Institute for In Vitro Sciences, Gaithersburg, MD 20878. E-mail: jharbell@iivs.org

Controls are a fundamental requirement in the design of any experiment or assay, whether the test system is a whole organism or an isolated part. *In vitro* studies allow one to focus on a select subset of dependent variables (end points) and provide much greater control over the independent variables than might be possible in the whole organism. This reductionist approach also limits the number of possible mechanisms by which a material might impact on the test system. Since the end point(s) determine the mechanism(s) that may be assessed, their selection is critical. Two questions must be addressed in any study: 1) which mechanisms can be identified and measured by the end points selected and 2) have all of the expected/unexpected independent variable been identified and held within an acceptable limits over time and replicate assays. The use of concurrent assay controls (positive and negative) and appropriate reference material provides the means to answer both questions. The reference materials are selected to link the assay end point(s) with an expected mechanism of action in the cell or tissue. The goal may also be to link the *in vitro* end point with a manifestation of action in the whole organism. They should always be used in assay development and when new end points are proposed. The assay controls focus on the stability of the independent variables, and thus stability of the system, over time. They link experiments within a larger study and are essential if data are to be interpreted across laboratories. This presentation will focus on the need for controls in all studies and practical methods for designing them into *in vitro* studies.

W-4

Fundamentals of Classical Cryopreservation. L.E. MCGANN. Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta CANADA T6G 2R8. Email: locksley.mcgann@ualberta.ca

Protocols for cryopreservation of cells in suspension have, historically, been developed empirically. Understanding of cellular responses at low temperatures subsequently evolved, including influences of the composition of the residual solution, osmotic stresses, solute toxicities, and intracellular freezing. This understanding allows simulations of osmotic responses during addition and removal of cryoprotectants, and during cooling and warming. Based on measured osmotic permeability parameters and their temperature dependencies, simulations are now used to optimize cryopreservation protocols by minimizing osmotic injury and toxicity during addition and removal of cryoprotectants, and by developing protocols to avoid conditions leading to intracellular freezing and other sources of low-temperature injury. Cell-matrix and cell-cell interactions significantly alter low-temperature responses, resulting in major post-thaw consequences for cell viability and tissue function. Natural and biosynthetic articular cartilage and corneas, and even monolayers of cells on a substrate demonstrate the importance of conditions leading to extracellular and intracellular ice nucleation, the amount and locations of ice, the distribution of electrolytes as ice forms, and the consequences of osmotic volume changes. As engineered cells and biosynthetic tissues become increasingly important in science, medicine, and industry, research in cryobiology is challenged to extend our understanding and develop effective and robust preservation technologies.

W-5

Apoptotic Proteolytic Cascades Contribute to Cryopreservation-induced Delayed-onset Cell Death. JOHN M. BAUST, Robert Van Buskirk, and John G. Baust. Institute of Biomedical Technology, State University of New York, Binghamton, NY 13902-6000. BioLife Solutions, Inc., Ewing, NJ 08628. E-mail: jmb46@cornell.edu

Recent identification of the phenomena of cryopreservation-induced delayed-onset cell death (DOCD) has begun to provide an avenue of explanation for the observed biologic failure following cryopreservation (CP). (Baust, 2001) Further, investigations have revealed that apoptosis, programmed cell death, plays an integral role in the execution of DOCD following CP. Identification of apoptotic involvement in DOCD led us to investigate the activity of the proteolytic apoptotic pathway following CP. We hypothesized that a CP-dependent up-regulation in the caspase cascade contributes to DOCD. We further hypothesized that the reported improvements in CP efficacy through the utilization of HypoThermosol (HTS), an intracellular-type carrier medium, was due to a decrease in caspase activity following CP. We now report on the up-regulation of caspase-3 following CP and that the utilization of HTS as the carrier medium during CP significantly reduces post-thaw caspase-3 activity. Human Dermal Fibroblast cells were subjected to a controlled rate freezing protocol in media + 5% DMSO or CryoStor CS5 (HTS + 5% DMSO). Cells were incubated at 10 C (10 min) in the preservation media, cooled at 1 C×min-1 to -80 C and quenched in LN2. Following storage, cells were thawed to 37 C and directly plated in FGM (1:11 volumes). Post-thaw viability of the cells was assessed daily using a non-invasive metabolic indicator, alamarBlue, and a nucleic acid stain, SytoDye-24. Differential activity of caspases following cryopreservation was investigated via post-thaw, time course western blot and protease-activity analysis. Results: 1.) Utilization of CryoStor CS5 resulted in an increase in cell survival over that of media + 5% DMSO (68% vs. 30%), 2.) western blot analysis revealed a 2 fold enhancement in active-caspase-3 expression following CP with peak activity observed between 18-24 hours post-thaw, 3.) caspase-3 protease-activity analysis verified western blot analysis, revealing a peak in protease activity at 24 hours post-thaw. These studies illustrate that activation of the proteolytic caspase cascade plays an integral role in the execution of CP-induced DOCD. Specifically, caspase-3 activation following CP contributes significantly to CP failure. Further, utilization of CryoStor CS5 resulted in a significant reduction in caspase-3 activity and a corresponding increase in CP efficacy. These data further illustrate that the control of apoptotic cell death, particularly caspase activity, may facilitate further enhancement in cell survival following CP. (Research funding provided by NIH, NSF, and BioLife Solutions, Inc. JMB is an International Foundation for Ethical Research Graduate Fellow)

W-6

Cryopreservation with the Avoidance of Ice. MICHAEL J. TAYLOR. Organ Recovery Systems, Inc., Port City Center, Charleston, SC 29403. E-mail: mtaylor@organ-recovery.com

Techniques for the cryopreservation of a wide variety of cells and biosystems have been established since the early discoveries of the crucial role for cryoprotective additives in the 1950's. A considerable degree of understanding of the mechanisms of freezing injury and its prevention have ensued and it has long been anticipated that these cryobiological techniques would also provide the means for long-term storage of more complex tissues and possibly organs. However, it is now recognized that organized multicellular tissues are subject to additional mechanisms of cryoinjury and the destructive effect of extracellular ice, in particular, must be minimized by reducing or eliminating ice crystallization during cryopreservation. The emerging field of tissue engineering is an interdisciplinary field that has largely trivialized the importance of cell and tissue storage for extended product shelf-life. Clearly, the problems inherent in the cryopreservation of natural tissues will also apply to engineered tissues, but in addition the relationships between the biological and synthetic component of a tissue construct may impose special considerations for successful cryopreservation. The amount and location of extracellular ice formation within the system is likely to be a critical determinant of tissue integrity and survival. The basic principles of low temperature storage of biological systems will be reviewed in relation to the challenges of cryopreserving multicellular tissues. The focus will be upon new technologies developed to minimize the hazards of ice crystallization using both vitrification and molecular ice control methods. Vitrification involves the stabilization of the biological system in the glassy (vitreous) state without the inherent problems associated with water crystallization and so-called solution effects injury. This technique has been successfully applied to the long-term preservation of a variety of cell-types but only recently has it been shown to provide an effective method of cryopreservation in more complex tissues that have proved refractory to conventional cryopreservation involving freezing.

W-7

Use of Intracellular Sugars for Stabilization of Mammalian Cells in Dried State. Tani Chen[1], Jason Acker[1], Alex Fowler[2], Hagan Bayley[3], MEHMET TONER[1]. [1]Massachusetts General Hospital/Harvard Medical School/Shriners Hospital for Children, The Center for Engineering in Medicine, Boston, MA 021141; [2]University of Massachusetts-Dartmouth, Mechanical Engineering, Dartmouth, MA 027472; and [3]Texas A&M University, Medical Biochemistry and Genetics, College Station, TX 778433. Email: mtoner@sbi.org

With recent advances in tissue engineering, cell transplantation, and genetic technologies, the living cell is becoming an important therapeutic tool in clinical medical care. Successful long-term storage of living cells is critical to the success of these emerging approaches. Here, we report that the introduction of low concentration of small carbohydrate sugars such as trehalose can greatly improve the survival of mammalian cells in storage. Using a genetically engineered mutant of Staphylococcus aureus alpha-hemolysin to create pores in the cellular membrane, we were able to successfully load trehalose into cells. We then showed significant beneficial effects of small amounts (0.1 to 0.2M) of trehalose loaded into mammalian cells during a freeze-thaw cycle to liquid nitrogen temperature (i.e., -196°C). More recently, we have focused on our efforts to dry storage of mammalian cells in sugar glasses. Differential scanning calorimetry was used to study the glass transition temperature in cells containing intracellular trehalose. Our results show higher glass transition temperatures and enhanced stability of the cytoplasm due to the presence of trehalose, compared to cells without intracellular trehalose. In addition, initial experiments show that cell survival depends on the glass transition temperature, as well as the storage temperature and the final moisture content. Thus, these initial studies provide evidence that simplified and widely applicable protocols in dry state at ambient temperature for long-term storage of living cells may be possible using sugars as intracellular stabilizers.

I-1000

Stem cells from insect midgut cultures differentiate in response to two new peptides from insect hemolymph. M.J. LOEB and H. Jaffe. Insect Biocontrol Lab, USDA, Beltsville MD 20705; and LNC-NINDS Protein/Peptide Sequencing Facility, NINDS, National Institutes of Health, Bethesda, MD 20892. Email: loebm@ba.ars.usda.gov

Two years ago, we reported that peptide factors from conditioned medium in which *Manduca sexta* midgut cells were grown induced isolated stem cells from *Heliothis virescens* midgut to differentiate *in vitro* to mature columnar and goblet gut cells. These peptides were identified as fragments of fetuin, one of the constituents of the bovine serum albumin that was essential for culturing these cells. We report here that two completely different peptides have been isolated from hemolymph of newly pupated *Lymantria dispar*. These peptides similarly induce differentiation of *H. virescens* midgut stem cells. Like fetuin, hemolymph was only active after treatment with chymotrypsin. Partially purified hydrolyzate was subjected to 3 successive RP-HPLC separation steps using Vydac, Zorbax and YMCODS columns. Thirty second fractions were bioassayed in groups of 5; active groups were then bioassayed singly. Well- separated peaks were subjected to Edman degradation, revealing nonapeptides EEVVKNAIA and ITPTSSLAT. No matches to known peptides were found in the BLAST database. Synthetic peptides were maximally active *in vitro* at 10^{-6} and 10^{-9} M, respectively. Lepidopteran midgut cells isolated in culture are able to maintain homeostasis or adjust proliferation and differentiation to culture conditions. Various combinations of the 4 peptides incubated with *H. virescens* stem cells were either no more effective than 1 peptide alone, more stimulatory, or inhibitory to differentiation, suggesting that local secretion of combinations of the peptides could regulate midgut differentiation *in vivo*.

I-1001

Differences in Production Levels of HzSNPV in Low and High Passages of the *Heliothis virescens* Cell Line HvAM1. C.L. GOODMAN, A.H. McIntosh, J.J. Grasela, S.G. Saathoff, and C.I. Ignoffo. USDA, ARS, Biological Control of Insects Research Laboratory, Columbia, MO 65203. Email: goodmanc@missouri.edu

Baculoviruses are Order-specific viruses which may be used to control important pest insects, especially lepidopteran larvae. As such, it is crucial to determine whether or not insects can become resistant to these viruses, either to wild-type or recombinant strains. To determine this, we used both *in vivo* and *in vitro* approaches, with data from the latter studies being presented here. Larvae of the tobacco budworm, *Heliothis virescens*, are known to be highly susceptible to infection by the singly-enveloped nucleopolyhedrovirus, HzSNPV. Conversely, the *H. virescens* cell line, HvAM1, has been shown to display a low susceptibility to infection by HzSNPV. We initially decided to use this cell line to attempt to develop an HzSNPV-sensitive population for its use in comparative studies with the HzSNPV-resistant cell population. In the process of evaluating the virus production levels of different HvAM1 passages, we discovered that a high passage of HvAM1 (which had been adapted to a different culture medium) contained a significantly higher percentage of HzSNPV-sensitive cells than did a low passage from this same cell line. These data as well as pertinent cell line characterization data, will be presented and implications for virus-resistance mechanisms will be discussed.

I-1002

Application of DNA Microarray Technology for Gene Discovery and Expression Analysis in a Non-model Organism. SHIRLEY A. POMPONI, Robin Willoughby, and Christopher G. Russell. Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1 North, Fort Pierce, Florida 34946 (S.A.P. & R.W.); and Research Genetics, Inc., 2130 Memorial Parkway SW, Huntsville, AL 35801. (C.G.R.). E-mail: pomponi@hboi.edu

Marine sponges are known to produce thousands of biologically active natural products with potential as pharmaceuticals and other bioproducts. As such, they are targets for cell culture. DNA microarrays are being used as tools for the study of genetic homology, differential expression, and gene discovery, and for elucidation of previously unknown structural and functional features of a marine invertebrate genome. The shallow water sponge *Axinella corrugata* (=*Teichaxinella morchella*) is the primary model. Marine sponge cDNA was hybridized to human Gene Filters (Research Genetics, Inc.). Widespread and specific hybridization is seen without altering standard stringency conditions. Even in the absence of a cell line, and in the presence of considerable cell type heterogeneity, outlying expression ratios that exceed the background of variability have been detected. Seven genes appear to be predictably up-regulated in the marine sponge in response to treatment with serum-containing nutrient-rich medium and/or the mitogen phytohemagglutinin. This work demonstrates the utility of microarrays for exploiting existing knowledge of model genomes for the investigation of non-model systems.

P-1000

Expression of a GFP Fusion Marker Under the Control of Three Constitutive Promoters and Enhanced Derivatives in Transgenic Grape. Z. LI, S. Jayasankar, and D.J. Gray. MREC-IFAS, University of Florida, 2725 Binion Road, Apopka, FL 32703.

Activity of three constitutive promoters and enhanced derivatives in transgenic grape (*Vitis vinifera* L. cv. Thompson Seedless) was characterized using a bifunctional fusion marker containing the enhanced green fluorescent protein (EGFP) and neomycin phosphotransferase (NPTII) genes. Relative differences in transient GFP expression and stable transformation efficiencies were used to compare promoter activity. Expression patterns in transformed somatic embryos revealed that the ACT2 promoter from *Arabidopsis thaliana*, previously shown to be a strong constitutive promoter in *A. thaliana* and other species, failed to promote strong expression in grape. In contrast, a promoter isolated from cassava vein mosaic virus (CsVMV) supported high levels of transgene expression equivalent to those achieved using an enhanced double CaMV 35S promoter. Duplication of the 5′ -upstream enhancer region of the CsVMV promoter further enhanced its ability to increase transgene expression. However, the pattern of transgene expression driven by these two viral promoters was significantly different at the whole plant level. The enhanced double CaMV 35S promoter was highly active in most tissues and organs including roots, mature leaves, shoot apices and lateral buds. In contrast, the CsVMV promoter and its double enhancer derivative induced relatively weak expression in these tissues. Our results suggest that activity of the CsVMV promoter, in contrast to the CaMV 35S promoter, was under developmental regulation in transgenic grape plants as compared to the CaMV 35S promoter.

P-1001

High Efficiency Transformation of Egg Plant (Solanum melongena L.) by Agrobacterium tumefaciens. G. FRANKLIN and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, INDIA. Email: gfrank@mcbl.iisc.er

An efficient method of producing transgenic eggplant (*Solanum melongena* L.) *via Agrobacterium*- mediated genetic transformation was developed. Explants were transformed by cocultivation with *Agrobacterial* strain LBA4404. The strain harbours a binary vector pBAL2 carrying the reporter gene GUS intron (GUS-INT) and the marker gene neomycin phosphotransferase (NPTII). Transformation efficiency depends on an efficient regeneration system, in addition to other parameters. Hence regeneration potential of different explants was studied in detail and the most optimum concentration was used in the present study. Among the different combinations of TDZ and NAA tested, 0.1 mg/l of TDZ and 0.2 mg/l NAA in the medium influenced efficient regeneration of shoots via indirect organogenesis. Callus induction and shoot regeneration occurred subsequently in the same media. The frequency of transgenic calli formation was better with cotyledonary explants compared to leaf explants. Shoot buds elongated in the same media in 3-4 weeks time. The putatively transformed shoots were harvested and placed directly for rooting on soil (soilrite) watered with sterile water containing 100 mg/l kanamycin. Molecular analysis of the field established plants was carried to confirm the transgenic nature from the genomic DNA isolated. The presence of GUS and NPTII genes in the transgenic plants was verified by histochemical GUS assay and PCR analysis respectively. Integration of T-DNA into the genome of putative transgenics was further confirmed by Southern blot analysis. GUS hitstochemical assay was also positive in the T1 plants. A total of 124 transgenic plants were raised in pots and mature fruits were collected. Progeny analysis of these plants showed a pattern of classical Mendelian inheritance for both NPT11 and â-glucuronidase (GUS) gene expression.

P-1002

MicroTom---A Model Functional Genomics Assay. YINGHUI DAN, Hua Yan, Tichafa Munyikwa, Jimmy Dong, Bei Zhang, Linda K. Lahman, and Caius Rommens. Monsanto Company GG4B, 700 Chesterfield Parkway North, St. Louis, MO 63198. Email: yinghui.dan@monsanto.com

MicroTom is a miniature-dwarf-determinate *Lycopersicon esculentum* cultivar, originally bred for home gardening purposes. It differs from standard tomato cultivars by only two recessive genes, which provide the dwarf phenotype to MicroTom. MicroTom shares features with Arabidopsis that make it successful as a model system, i.e., small size (up to 1357 plants/m^{-2}), short life cycle (70-90 days from sowing to fruit ripening), well-characterized genetics, and small DNA content per haploid genome. A model functional genomics assay, composed of three elements which are genomics, plant transformation, and plant pathology technologies, has been developed in order to use MicroTom to accelerate gene discovery for fungal disease control. Large number of candidate genes for disease resistance have been identified, cloned, and constructed into vectors using genomics approaches. An efficient *Agrobacterium*-mediated transformation protocol has been developed, and this protocol has been used for transgenic plant production at industrial scale. Forty-seven candidate genes were introduced into MicroTom using the protocol. A large transgenic R_1 population containing the genes are ready to be challenged against pathogens. The focus in this presentation will be on elucidation of the concept of this assay associated with identification of disease resistance genes, plant transformation development, and gene function evaluation as it applies to the concept.

P-1003

Tomato Fruit with Enhanced Calcium Nutrition. S. H. PARK, K. D. Hirschi, J. E. Park, and R. H. Smith. Vegetable & Fruit Improvement Center, Texas A&M University, College Station, TX 77845. Email: rsmith@tamu.edu

The benefits of elevated calcium in tomato fruit could include enhanced nutritional value for calcium supplementation, potential resistance to pathogen infection during handling of the fruit, and control of blossom end rot due to low calcium in fruit. Tomato, *Lycopersicon esculentum* Mill., cultivars have been transformed using *Agrobacterium tumefaciens* with a gene for Ca^{2+} accumulation, CAX1 (Calcium Exchanger 1) from *Arabidopsis*. The effect of several promoters including: E8, a fruit specific promoter, 35S a constitutive promoter and cdc2, a cell division cycle promoter, will be reported. Calcium expression in both fruit and vegetative plant parts will be evaluated. Initial work on two model system tomato cultivars, Micro-Tom and Red Cherry has resulted in transgenic plants. The primary plants exhibit calcium deficiency symptoms using the 35S, and cdc2 promoters; however, vegetative and fruit analysis establishes elevated calcium levels. Fruit has been analyzed with a 150% increase in calcium levels. Data on transgenic commercial processing and fresh fruit cultivars of tomato will be presented.

P-1004

Transformation of Multiple Genes into Soybean (*Glycine max* (L.) Merrill) by Co-bombardment and by a 6-Gene Cluster Plasmid. M.A. SCHMIDT, B.J. Artelt, and W.A. Parrott. University of Georgia, Dept. of Crop and Soil Sciences, Athens, GA 30602-7272. E-mail: schmidt@uga.edu

The trend is for cultivars to have multiple traits "stacked" together. In addition, many agronomically important traits are polygenic. The manipulation of such traits will likely involve the redirection of complex metabolic or regulatory pathways. A prerequisite for the alteration of such pathways is a gene transfer methodology that will allow for simultaneous multiple gene transfers and the insurance of subsequent stable expression and inheritance in succeeding generations. To investigate the feasibility and the properties of multiple gene transfer into soybean, six genes were transformed into somatic embyrogenic soybean cultures via particle bombardment using a single 6-gene containing plasmid or a cocktail of 5 separate plasmids. The five plasmids used for cotransformation included plasmids that encode for (1) both hygromycin resistance and bialaphos resistance, (2) Beta-glucuronidase, (3) green fluorescent protein, (4) bleomycin resistance, and (5) kanamycin resistance. Co-transformation was performed using two different plasmid cocktails: an equimolar ratio of all plasmids and a 1:9 molar ratio of the hygromycin-encoding plasmid to the remaining 4 plasmids. The single 6-gene containing plasmid was pMECA derived and contained the same selectable marker and reporter genes. One week following bombardment, the cultures were placed under hygromycin selection and after an additional 8 weeks, hygromycin-resistant lines were isolated. Lines were deemed transgenic by the presence of the hygromycin gene as detected by PCR and/or Southern hybridization analysis. Three lines from the single 6-gene containing plasmid treatment, 5 lines from the eqimolar cocktail treatment and 3 lines from the 1:9 molar treatment have been isolated. Presently, only a T_0 line that was produced by the bombardment of the single 6-gene containing plasmid treatment was identified to contain all 6 transgenes. Of the transgenes analyzed thus far, the genes encoding for hygromycin resistance, kanamycin resistance, GFP and GUS are correctly expressed in this line. The first transgenic line produced, derived from the 1:9 cocktail treatment, has been carried through to the T_1 generation and progeny were determined to be transgenic by PCR analysis. The remaining lines are being analyzed to determine the frequency with which the 6 transgenes are present in the different transformation treatments.

P-1005

GFP Introduction, Expression, and Possible Toxicity in Soybean. K.M. Larkin, M. Buenrostro-Nava, and J.J. FINER. Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691. E-mail: FINER.1@OSU.EDU

The green fluorescent protein (GFP) has been used extensively in recent years as a scorable marker in a number of different organisms. Initial reports of poor expression and toxicity brought about development of improved forms of GFP which possess modified excitation and emission maxima, enhanced solubility, and modified codon usage. Toxicity problems were apparently minimized or eliminated with the exploitation of ER targeting signals, which directed and sequestered the potentially toxic protein to the lumen of the ER. An ER-targeted GFP (GFP5-ER) was introduced into soybean using Sonication Assisted *Agrobacterium*-mediated Transformation of proliferative embryogenic tissue. *Agrobacterium* strain EHA105 was used as the transformation vector and hygromycin resistance was used as the plant selectable marker. Although GFP was initially detected 4 days following inoculation, expression declined soon after and was not observed again until 10-14 weeks post-selection. GFP expression could not be followed from single cells but appears to spread rapidly in selected hygromycin-resistant tissue. GFP expressing clones that were recovered did not display solid or constitutive expression but appeared mottled. We believe that this tissue was not chimeric but showed variable GFP expression. Tracking of GFP-expressing soybean clones indicated that tissues, which expressed GFP at high levels, turned brown and senesced while portions of the same clone, that did not express GFP at high levels, continued to proliferate, with variable GFP expression. We believe that high levels of GFP expression is toxic to proliferative embryogenic soybean tissues and that use of this gene as a scorable marker should be carefully evaluated for each different target tissue. Efforts are underway in the laboratory to text different tissue-specific promoters with GFP and evaluate other fluorescent proteins, which are less toxic and may be more suitable for transformation studies in soybean.

P-1006

Use of Barley Endosperm-specific Hordein Promoters for Production of Recombinant Proteins in Transgenic Cereal Seeds. M.-J. CHO, B.B. Buchanan, and P.G. Lemaux. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: mjcho@nature.berkeley.edu.

Barley (*Hordeum vulgare* L.) hordeins are major seed storage proteins that accumulate in protein bodies of the developing starchy endosperm. In order to produce β-glucuronidase (GUS), green fluorescent protein (GFP) or wheat thioredoxin *h* (WTRX*h*) in transgenic cereal seeds, barley, wheat, rice, or maize was transformed with chimeric hordein-*uidA* (*gus*), -*gfp*, or -*wtrxh* gene construct with or without a hordein signal peptide sequence. Developmental and endosperm-specific expression of GUS and/or GFP was observed in T_1 seeds of barley, wheat and/or maize as determined by histochemical and fluorometric assays. Wheat thioredoxin *h* was also overexpressed in seeds of transgenic barley, wheat, and rice. Higher expression and activity of GUS and WTRX*h* were detected in transformed barley seed with the DNA constructs containing the signal sequence than those without the signal sequence; GFP expression is currently being assessed. All three transgenes driven by the endosperm-specific B_1- or D-hordein promoter were stably inherited and expressed in progeny of the all transgenic cereal plants tested. We conclude that the B_1- and D-hordein promoters can be used to engineer, and subsequently study, stable endosperm-specific gene expression in cereal plants and potentially to modify cereal seeds through genetic engineering.

P-1007

Herbicide and Insect Resistance in Transgenic Rice. S. H. PARK, K. D. Hirschi, J. E. Park, and R. H. Smith. Vegetable & Fruit Improvement Center, Texas A&M University, College Station, TX 77845. Email: rsmith@tamu.edu

Transgenic rice (*Oriza sativa* L.) containing two agronomically important genes was generated using *Agrobacterium* LBA4404 with an additional virulence plasmid, *virG*(pTiBo542)/*virE1virE2*(pTiA6). The plants were transformed with phosphinothricin acetyl transferase (*pat*) gene for herbicide resistance and *Bacillus thuringiensis* (*Bt*) crystal insecticidal protein gene for insect resistance. Three different sets of primary plants expressing the *pat* and *Bt* genes, the *pat* gene and fragmented (nonfunctional) copy of the *Bt* gene, or the *pat* gene only were produced. Stable integration, expression, and transmission of the transferred genes in T_0 and T_1 generation plants were confirmed by both molecular analysis and phenotype expression. The herbicide application test of the progeny from the three sets of primary plants showed that the transferred *pat* gene was stably expressed in the T_1 generation. The insect feeding bioassay with T_1 generation plants conferring resistance to herbicide established that the transgenic plants having a complete *Bt* gene were toxic to tobacco budworm (*Heliothis virescens*) larvae. The insect feeding bioassay and herbicide application test results were clearly correlated with the molecular analysis.

P-1008

Generation and Evaluation of Transgenic Tall Fescue Plants. ZENGYU WANG, Jeremy Bell, Deane Lehmann, Megann Scott, Chungkyoon Auh, Paul Dowling, and Andrew Hopkins. Forage Biotechnology Group, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. E-mail: zywang@noble.org

Tall fescue (*Festuca arundinacea*) is the predominant cool season forage grass in north America. An efficient plant regeneration system has been established for this monocot species based on single genotype-derived embryogenic suspension cultures. Transgenic tall fescue plants have been obtained by biolistic transformation of suspension cells using selectable marker and reporter genes. Hygromycin phosphotransferase gene (*hph*) has been used as selectable marker. Sets of transgenic tall fescue plants have been transferred to the field and are being evaluated for the second year. In order to study pollen flow for this outcrossing species, seed-derived control plants were planted around the transgenic plot in eight directions. Other *Festuca* species were also planted in the field to study the possibility of crossings with transgenic plants. Transmission genetics of the introduced foreign genes as well as agronomic performance of the transgenic plants and their progenies will be studied. Since digestibility of forage grasses is one of the major limitations on animal productivity, efforts have been made to improve digestibility of tall fescue by down regulation of lignin biosynthesis. Genes involved in lignin biosynthesis (CAD, COMT) have been isolated from tall fescue. Transgenic tall fescue plants carrying sense and antisense lignin biosynthetic genes have been generated and are being characterized.

P-1009

Plant Regeneration and Genetic Transformation of Russian Wildrye. JEREMY BELL, Deane Lehmann, Megann Scott, Andrew Hopkins, and Zengyu Wang. Forage Biotechnology Group, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401.

Russian wildrye (*Psathyrostachys juncea*) is an important cool season forage grass which showed potential to be grown in the Southern Great plains. An efficient plant regeneration system has been established for this monocot species based on single genotype-derived embryogenic suspension cultures. Large numbers of green plants were regenerated from the established suspension cultures. These regenerants were transferred to the field for the evaluation of somaclonal variation. Transformation parameters were partially optimized by transient expression of the *gusA* gene. In order to determine the lethal dose for selection of transformed cells, a dose response experiment was performed by exposing suspension cells to different levels of hygromycin. For stable transformation, hygromycin phosphotransferase gene (*hph*) has been used as selectable marker. Transgenic Russian wildrye plants have been produced by biolistic transformation of suspension cells using selectable marker and reporter genes. Sets of transgenic Russian wildrye plants have been transferred to the field. Transmission genetics of the introduced foreign genes will be studied.

P-1010

Identification of a Highly Transformable Wheat Genotype for Mass Production of Fertile Transgenic Plants. A. PELLEGRINESCHI, L. M. Noguera, S. McLean, B. Skovmand, R.M. Brito, L. Velazquez, R. Hernandez, M. Warburton, and D. Hoisington. Applied Biotechnology Center, CIMMYT, Apdo Postal 6-641, 06600 DF. MEXICO, E-mail: A.Pellegrineschi@cgiar.org

The efficiency of wheat biolistic transformation systems strongly depends on the bombardment parameters, the conditions of the donor plant, and also the plant genotype chosen for the transformation process. This paper analyzes the transformation efficiency of the 129 wheat sister lines generically called "Bobwhite". A number of factors influencing the transformation were examined, such as the ability to produce embryogenic callus, the regeneration in selection medium and the overall transformation performance. Of the 129 genotypes evaluated, eight demonstrated transformation rates above 60% (60 independent transgenic events for 100 immature embryos bombarded). Among the eight genotypes identified, we studied agronomic characteristics such as earliness, in order to identify the most adaptable line(s) for different lab conditions and we identified the Bobwhite SH 98 26 as the "super transformable" wheat line.

P-1011

Desiccation of Agrobacterium-inoculated Precultured Plant Tissues Significantly Enhances T-DNA Delivery, and Subsequently Increases Stable Transformation in Wheat. M. CHENG, T. Hu, J. Layton, C.-N. Liu, and J.E. Fry. Monsanto, Mystic, CT 03655. E-mail: ming.cheng@monsanto.com

Factors influencing the *Agrobacterium*-mediated transformation of both monocotyledonous and dicotyledonous plant species have been widely investigated. These factors include manipulating: *Agrobacterium* strains and plasmid, growth conditions for *vir* gene induction, the plant genotypes, inoculation and co-culture conditions, and the selection agents and their application regime. With the understanding of these factors, *Agrobacterium*-mediated transformation has been achieved recently in a wide range of the Gramineae crop species such as rice, maize, barley, and sugarcane, which were previously considered outside the host range of *Agrobacterium*. Genetic transformation of wheat mediated by *Agrobacterium tumefaciens* has been achieved in our lab. The transformation efficiency in our initial experiments was quite low, even though a large number of transgenic plants have been produced. The major problems associated with the system were either low T-DNA delivery in some explants or poor plant cell recovery after *Agrobacterium* infection. This presentation will discuss a desiccation treatment on the inoculated plant tissues precultured on plant tissue culture medium during co-culture. This treatment greatly enhances the T-DNA delivery in both monocotyledonous and dicotyledonous crops, especially in wheat and subsequently increases the stable transformation efficiency in wheat. The transgene integration and co-expression with the new transformation system will also be discussed.

P-1012

Constitutive Expression of an Endogenous Antifungal Protein Alpha-hordothionin in Transgenic Barley. JIANMING FU(1), Puthigae Sathish(1), Maria L. Federico(1), Heidi F. Kaeppler(1), Ron Skadsen(2) (1) Agronomy Dept. of Wisconsin-Madison, Madison, WI 53706; (2) USDA/ARS, Cereal Crops Res. Unit, 501 Walnut St., Madison, WI 53705. E-mail: jianmingfu@facstaff.wisc.edu

We have developed a system to constitutively express a gene coding for an endogenous antifungal protein alpha-hordothionin (HTH) in transgenic barley. HTH is toxic to *Fusarium graminearum* (*F.g.*, the pathogen that causes barley and wheat scab disease) at a concentration of 5.0 μg/ml, as demonstrated by our toxicity assay using purified HTH from barley endosperm. Native HTH does not protect barley from infection, however, because F.g. colonizes the lemma and palea surrounding the endosperm and HTH is confined to the endosperm. A full-length HTH cDNA clone (HTH-1) synthesized from mRNA of developing barley endosperm, and an inhibitory DNA sequence at the 5′-coding region of the gene was identified and deleted. The optimized cDNA (*HTH-2*) was fused between a maize ubiquitin promoter and a *nos* termination sequence, and then transformed back into barley via particle bombardment. Nine out of eleven independent transgenic callus lines have been regenerated, and the regenerated plants are growing in the greenhouse. Stable transformation was confirmed by PCR and Southern blot analysis. The mRNAs of the transgene were detected by RT-PCR and northern blot analysis. Research to detect transgene-encoded HTH protein and to test *Fusarium* resistance of the transgenic plants is underway. Grain products containing transgene-derived HTH should be safe for consumption since HTH is a naturally occurring dietary protein that is located in barley endosperm.

P-1013

Expression of Maize *Rp1-D* Rust Resistance Gene in Transgenic Maize and Wheat. MARTIN STEINAU, Scot H. Hulbert, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506. E-mail: trick@antpath.ksu.edu

The maize *Rp1-D* gene was previously identified as a putative R gene to promote a hypersensitive response and consequent resistance to common leaf rust *Puccinia sorghi*. To confirm this function and for further analysis, the gene was transformed into rust susceptible maize lines via biolistics. Besides the native *Rp1-D* full-length clone, a second construct was used placing the ORF under the control of the strong and constitutive maize ubiquitin promoter. Several independent fertile transgenic lines have been recovered from tissue culture and were analyzed by PCR and southern blot hybridization and FISH. Copy numbers ranged from 1 to over 14. RT-PCR revealed a range of *Rp1-D* transcription levels from these transgenic clones. In bioassays, T_1 plants were challenged with *Puccinia sorghi* isolates and the different degrees of observed resistance were linked to the expression levels. In addition, it was investigated if *Rp1-D* could also be functional in wheat and promote potential resistance to related Puccinia sp. Therefore the gene was transformed into 'Bobwhite' callus explants using particle bombardment. Molecular analysis indicated integrated transgene copy numbers between one and six. RT-PCR and Northern Blot analysis identified two lines transcribing *Rp1-D* mRNA. Transgenic lines were also crossed to the hypersensitive variety 'Thatcher' and bioassays with wheat leaf rust isolates were performed on these plants.

P-1014

Transformation of Peanut with Truncated Nucleocapsid Protein Gene of Tomato Spotted Wilt Virus Gene in Cultivated Peanut (*Aarachis hypogaea* L.) Using Particle Bombardment. H.Y. YANG[1], H. Pappu[2], and P. Ozias-Akins[1]. [1]Department of Horticulture, [2]Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31794. E-mail: ozias@tifton.cpes.peachnet.edu

Tomato spotted wilt virus (TSWV) is a devastating disease of many crops including peanuts. Because the virus has a broad host range and is carried and spread by ubiquitous thrips, disease control management is very difficult by traditional means. Developing new cultivars with adequate resistance to spotted wilt still presents a big challenge in combating this disease. Since natural resistances in peanut germplasm are limited, a genetic engineering approach appears to have great potential for resistance enhancement to spotted wilt virus. In a continuous effort to achieve TSWV protection, we are utilizing an approach of RNA-mediated resistance in which we engineered peanut with transformation vectors that were intended to produce mRNA but not protein. The constructs contained only one half of the nucleocapsid protein gene sequences derived from a peanut isolate. The DNA fragment was cloned into the *Nco* I site of pAPCHIII containing a hygromycin resistance gene, yielding two vectors, PAPCH-NP411F and pAPCH-NP411R, with one having the 1/2 N gene in forward and the other in reverse orientation. Peanut somatic embryos were bombarded with either one or both plasmids combined. After 8-10 weeks in liquid selection medium following bombardment, hygromycin-resistant cell lines were observed, indicating that the NP gene was successfully introduced into peanut somatic embryos. Using the nucleocapsid protein gene-specific primer pair 94-265 and 94-266p, the transgene was found in peanut plants regenerated from somatic embryo tissues selected on hygromycin medium. Southern blot analysis confirmed the stable integration of the NP gene in the peanut genome. Expression of the truncated nucleocapsid protein gene was detected in both forward and reverse transgenic plants by Northern blot analysis with an N gene-specific probe. As expected, ELISA could not detect the production of the nucleocapsid protein in the transgenic plants. Seeds from both versions of constructs were harvested. Progeny production is under way that will be used for virus resistance screening.

P-1015

Production of Fertile Transgenic Soybeans with Putative Enhanced Disease Resistance. WOJCIECH ORNATOWSKI[1], William Schapaugh[1], S. Muthukrishnan[2], Timothy C. Todd[3], and Harold N. Trick[3], [1]Department of Agronomy, [2]Department of Biochemistry, [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. E-mail: TRICK@PLANTPATH.KSU.EDU

Genetic transformation is one approach to enhance disease resistance in crop plants especially where there is only limited or no known resistance available. Protection against charcoal rot, where there is no known resistance in the current germplasm and soybean cyst nematode resistance, where there is three known sources of resistance being used are two examples. Our laboratory has been transforming soybean with several antifungal genes including chitinases and glucanases and with a synthetic chitinase gene having putative nemacidal and insecticidal properties. Somatic embryogenic tissue of soybean cultivars "Chapman" and "Jack" were transformed with a hygromycin resistant gene (*hpt*) and either a rice chitinase gene (*chi11*) gene or a *Manduca sexta* chitinase gene (*msc*). A total of 24 independent transformants (15 transformed with the rice *ch11* chitinase gene and nine transformed with *msc* gene) have been confirmed by the polymerase chain reaction (PCR) to contain our selectable (*hpt*) marker gene and the gene of interest. These putative clones have been placed on maturation media for further development. Six of these clones engineered with the *ch11* gene and three with the *msc* gene were regenerated into seedlings and have been transferred into soil. The integration, inheritance and expression of the *ch11* and *msc* c genes have been confirmed by molecular analysis of T_0 and T_1 soybean transgenic plants. Results of bioassays will be discussed.

P-1016

Transformation with a Pathogen-inducible Stilbene Synthase Gene for Increased Fungal Resistance in Papaya. Y. Judy Zhu[1], C. S. Tang[2], Maureen Fitch[3], and Paul Moore[3]. [1]Hawaii Agriculture Research Center, 99-193 Aiea Heights Drive, Aiea, Hawaii 96701; [2]Department of Environmental Biochemistry, College of Tropical Agriculture and Human Resources, University of Hawaii, Hawaii 96822; [3]U.S. Department of Agriculture, Agriculture Research Service at Hawaii Agriculture Research Center, Hawaii 96701.

Papaya, one of the most important fruit crops in the tropics, is susceptible to a variety of pathogens including fungi, bacteria and viruses. Simultaneous control of both PRV and fungal diseases would decrease dependence on fungicides and significantly improve fruit quality. Phytoalexins have been shown to be important natural components in the defense of plants against fungal infection. We have obtained a transformation construct from Bayer AG that contains the *stilbene synthase* gene *(vst1)* from grapevine under control of its own inducible promoter and a hygromycin-resistance gene under the control of a CaMV35S-promoter. The precursor molecules for the formation of hydroxy-stilbenes, malonyl-CoA and p-coumaroyl-CoA are both commonly present in plants. Furthermore, using a gene with a pathogen-inducible promoter means that stilbene synthase will be expressed only at a low basal level in transgenic plants unless there is a pathogen attack. Following a transitory rise in expression, the expression is expected to return to a low level when the pathogen fails to establish. We conducted *in-vitro* pathogen inhibition assay to prove the stilbene will inhibit papaya fungi. Stilbene at 1.0 mM in V8 agar culture medium inhibited mycelia growth of P. palmivora up to 50% of control. The compound was active against P. palmivora as low as 0.1 mM. Stilbene was not as effective against the anthracnose pathogen, Colletotrichum gloeosporiodes. We have transformed Kapoho and PRV transgenic SunUp using the particle gun transformation system. The presence of transgenes has been confirmed by PCR and Southern blot. Several lines of trasngenic plants were propagated in the tissue cultures for greenhouse assays.

P-1017

Transgenic Cassava for Resistance to African Cassava Mosaic Disease. N.J. TAYLOR and C.M. Fauquet. International Laboratory for Tropical Agricultural Biotechnology (ILTAB), Donald Danforth Plant Science Center ILTAB-UMSL. CME 308, 8001 Natural Bridge Road, St. Louis, MO 63121. E-mail: ntaylor@danforthcenter.org

Cassava (*Manihot esculenta*) is a major source of dietary calories within the tropics and the first food crop in many low-income countries. Production of enhanced germplasm is considered the most effective manner in which to improve cassava yields. High heterozygosity and inbreeding depression frustrates conventional breeding and makes genetic engineering an attractive target in cassava. In this manner, traits for improved agronomic performance can be integrated directly into farmer-preferred genetic backgrounds. Procedures for the production of friable embryogenic tissues and their use as target tissues for transgene insertion have been developed and are now routinely employed at ILTAB to produce transgenic plants of the Nigerian cultivar TMS 60444. Both particle bombardment and *Agrobacterium* based gene transfer are being used to engineer cassava for elevated phosphate uptake and increased resistance to African cassava mosaic disease (ACMD), a geminivirus disease responsible for significantly depressing yields and impacting food supply throughout Sub-Saharan Africa. A pathogen-derived resistance strategy have been employed to regenerate transgenic cassava plants containing the defective interfering (DI) particle from African cassava mosaic virus. Challenge of transgenic plants by particle bombardment with infectious viral clones has shown this strategy to impart elevated resistance to cassava geminiviruses. Information will be presented as to the improved culture procedures being used to produce transgenic cassava plants at ILTAB, progress towards developing transgenic resistance to ACMD, genetic engineering of cassava for improved phosphate uptake, the transfer of these beneficial characteristics to agronomically important cassava cultivars and progress towards field trails of transgenic cassava in Africa.

P-1018

A study on the polyamine level during somatic embryogenesis development in *Vitis vinifera*. L. MARTINELLI[1], D. Bertoldi[1&2], A. Tassoni[2], E. Candioli[1], I. Gribaudo[3], N. Bagni[2]. [1]Istituto Agrario, 38010 San Michele a/Adige, Italy; [2]Dipartimento di Biologia, Università di Bologna, Italy; [3]Centro Miglioramento Genetico e Biologia Vite - CNR, Grugliasco, ITALY. E-mail: Lucia.Martinelli@ismaa.it

In the frame of a study aiming to enlighten developmental programs during regeneration in grapes, polyamine content (free and conjugated to hydroxycinnamic acids) and the biosynthetic enzyme activity were assayed during the crucial steps of somatic embryogenesis. Aliphatic polyamines, indeed, are growth regulators affecting plant growth and development both *in vivo* and during *in vitro* cultures, being involved in several morphogenic processes related to their action in cell division. The study was conducted on samples of callus, embryogenic callus, embryo at different stages and plantlets of *Vitis vinifera* 'Brachetto a grappolo lungo' and 'Chardonnay', induced from anthers and ovaries. Our results proved that polyamine content, referred to unit, was higher in the cv. Brachetto than in the cv. Chardonnay, and reached the higher levels in the fully-developed embryo stage. Besides, ornithine decarboxylase activity resulted higher than arginine decarboxylase, and during the development from callus to plantlets, both activities increased, reaching the maximum at this latter stage. Higher activity of both enzymes assayed in the small embryos rather than in the embryo with higher shape, was consistent with following polyamine accumulation. Authors wish to thank p.e. V. Poletti for technical support.

P-1019

Cre/lox Mediated Marker Gene Excision in Transgenic Crop Plants. LARRY GILBERTSON[1], Prince Addae[1], Charles Armstrong[1], Nelson Bernabe[2], Joanne Ekena[2], Greg Keithly[1], Mark Neuman[1], Virginia Peschke[1], Mike Petersen[2], Shubha Subbarao[1], Wanggen Zhang[1], Ken Barton[1]. [1]Monsanto Company, 700 Chesterfield Pkwy, Chesterfield MO 63198, [2]Monsanto Company, Agracetus Campus, 8520 University Ave. Middleton WI, 53562. Email: larry.a.gilbertson@monsanto.com

After the initial transformation and tissue culture process is over, selectable marker genes, which are used in virtually all plant transformation approaches, are not required for the expression of the gene of interest in the transgenic plants. There are several advantages to removing the selectable marker gene after it is no longer needed, such as recycling of selectable markers, and simplifying the gene cassette. We have tested the Cre/lox site-specific recombination system from bacteriophage P1 for the excision of selectable marker genes from transgenic plants in corn, wheat, soybean and cotton. Two strategies, crossing and autoexcision, have been developed to effectively remove marker genes from transgenic corn, wheat, soybean, and cotton plants. In the crossing strategy, plants expressing the Cre recombinase are crossed with plants bearing a construct in which the selectable marker is flanked by directly repeated lox sites. The efficiency of this strategy varies depending on the crop species, and can be nearly 100% efficient. In the autoexcision strategy, the cre gene, under the control of a heat shock inducible promoter, is excised along with the nptII marker gene. Our results show that a transient heat shock treatment of callus or plants is sufficient for inducing Cre and excising the cre and nptII genes. In corn, we have analyzed the effect of marker removal on the expression of an adjacent B.t. gene.

P-1020

In Vitro Regeneration of *Artemisia judaica* L. (Compositae) via Shoot Organogenesis and Somatic Embryogenesis. S.S.B. CAMPBELL, M.A. El-Demerdash, and P.K. Saxena. Department of Plant Agriculture, University of Guelph, Guelph, Ontario, CANADA, N1G 2W1. Email: psaxena@uoguelph.ca

Artemisia judaica is a perennial plant found in Egypt which has a reputation as a medicinal herb in the Arabian region. Two active constituents have been identified a flavone Cirsimaritin and a sesquiterpene keto-lactone Judaicin. Judaicin has been shown to have a more potent effect than digitoxin affecting acetylcholine and potassium chloride induced skeletal muscle contractions. Cirsimaritin shows antiviral and antibacterial activities as well as inhibitory affects on several mammalian enzymes. Traditional folk medicine uses this plant for the treatment of gastrointestinal disorders. *In vitro* methods for the production of sterile consistent plant material may help to further research into the biochemical composition of this species and the potential medical values. Wild harvested seeds were germinated in vitro and intact seedlings and etoliated hypocotyls were evaluated for regeneration potential. Naphthyleneacetic acid (NAA), benzylaminopurine (BAP), and thidiazuron (TDZ) induces a variety of effects on both hypocotyls and seedlings. Spontaneous shoot organogenesis was observed on seedlings which were cultured on a medium devoid of growth regulators. This response was decreased when seedlings were exposed to NAA and increased when exposed to BAP. Treatments of 10 uM BAP showed up to 80% of seedlings with multiple shoots. 10 uM NAA reduced the number of seedlings with multiple shoots from 50% seen on basal media to 20% of seedlings. Hypocotyls and seedlings exposed to TDZ underwent somatic embryogenesis via a callus phase following subculture on a basal medium. These data provide the foundation for further studies into the unique morphogenic responses and biochemical constituents of *Artemisia judaica.*

P-1021

A Role for Serotonin and Melatonin in Plant Morphogenesis. S.J. MURCH and P.K. Saxena. Department of Plant Agriculture, University of Guelph, Guelph, Ontario, CANADA, N1G 2W1. E-mail: psaxena@uoguelph.ca

St. John's wort (*Hypericum perforatum* L. cv Anthos) is a medicinal plant with historical and anecdotal evidence of efficacy as an anti-depressant. Recent research has demonstrated the recovery of radiolabel from tryptophan in serotonin and melatonin thereby indicating the biosynthesis of mammalian indoleamine neurohormones in leaf and stem tissues of St. John's wort. The objective of the current study was to assess the physiological role of melatonin and related indoleamines in plant morphogenic processes. In the initial experiments, two of the indoleamines, serotonin and melatonin, were supplemented to the culture medium. In subsequent research, six inhibitors of auxin and indoleamine metabolism, 2,3,5-triiodobenzoic acid, p-chlorophenoxyisobutyric acid, p-chlorophenylalanine, d-amphetamine, fluoxetine (Prozac(tm)), and methylphenidate (Ritalin(tm)) were included in a culture medium in the presence or absence of the auxin, indoleacetic acid. *De novo* shoot and root organogenesis and endogenous concentrations of auxins and indoleamines were quantified after 18-35 days. Increases in endogenous melatonin concentration corresponded with increased root formation, even in the absence of auxin accumulation. Conversely, the accumulation of serotonin was observed in the cultures that exhibited prolific shoot formation. These findings provide evidence of a role for the relative balance of serotonin and melatonin in plant regeneration.

P-1022

Induction of Somatic Embryogenesis and Shoot Organogenesis on Thin Cell Layers of African violet (*Saintpaulia ionantha*). J.M.R.VICTOR, S.J. Murch, and P.K. Saxena. Department of Plant Agriculture, University of Guelph, Guelph, Ontario, CANADA, N1G 2W1. E-mail: psaxena@uoguelph.ca

African violets have been sold commercially since the late 1800's and there are more than 296 patented varieties. Although numerous reports have described the regeneration of African violets on a variety of explants in various culture media, the responses have been primarily *de novo* shoot organogenesis. In the current study, thin cell layers and thin petiole slices of African violet (*Saintpaulia ionantha* Wendl. H. cv. Benjamin) were exposed to a cytokinin-supplemented growth medium. The regenerative responses of the sections varied with the growth regulator exposure. Culture of the sections on a medium devoid of growth regulators resulted in occasional shoot formation while exposure to a medium containing the auxin naphthyleneacetic acid resulted in prolific root formation. Thin cell layers and petiole slices exposed to naphthyleneacetic acid in combinations with benzylaminopurine resulted in *de novo* shoot organogenesis from the epidermal cells within 28-36 days. Petiole slices exposed to thidiazuron in the culture medium formed somatic embryos from the epidermal layers after 28-36 days of culture. Developing embryos formed individually covering the periphery and a suspensor was clearly visible between the maternal tissue and the developing embryos. No regeneration was observed in the cortical and pith regions of the explants. Embryos were removed and developed into intact plants *in vitro*. These data provide a new system for investigation of the factors involved in the induction of somatic embryogenesis and have commercial potential for the genetic improvement of African violets.

P-1023

Cryopreservation of Plumular Explants of Coconut (*Cocos nucifera* L.). P.T. LYNCH[1], R. Hornung[2], and R. Domas[2]. [1]Division of Biological Sciences, University of Derby, Kedleston Road, Derby DE22 1GB, UK. E-mail: P.T.Lynch@derby.ac.uk, [2]Plant Biotechnology Laboratories; Imperial College at Wye, T.H. Huxley School, Wye, Ashford, Kent, TN25 5AH, UK. E-mail: R.Hornung@ic.ac.uk

As a mainly outbreeding heterozygous plant, coconut seedlings can exhibit great variability in characters that cannot be evaluated until seedlings reach maturity. The ability to clone selected palms is of obvious significance. Recent development of somatic embryogenesis and plant regeneration from plumular explants, with more rapid development of calli bearing somatic embryos and greater frequencies of plant regeneration compared with calli derived from other explants, may provide a practical method of cloning. A more efficient method of cloning coconut also offers a potential for the development of a long-term *in vitro* means of conserving coconut germplasm by cryopreservation of plumular explants. Callus growth has been observed from plumules of ecotype Laguna Tall after cryopreservation using an encapsulation/dehydration protocol. Sucrose preculture and silica gel dehydration both significantly influenced the frequency of callus formation from non-frozen and frozen plumules. The greatest frequency of post-thaw callus growth occurred after incubation of the encapsulated plumules for 72 - 96 h in medium containing 0.75 M sucrose followed by desiccation over silica gel for 7 - 8 h. Post-thaw recovery rates in excess of 80% were recorded. Callus is currently being maintained to allow plant regeneration, but plant regeneration from non-frozen coconut plumule derived callus takes approximately 2 years.

P-1024

Changes in K, Mg, and Ca Levels in Embryogenic and Non-embryogenic Citrus Callus Subjected to Two Carbohydrate Sources for Somatic Embryogenesis Expression. S.C.C. Arruda1, M.A.Z. Arruda2, B.M.J. Mendes1, A.P.M. Rodriguez1*. Laboratório de Biotecnologia Vegetal, CENA, Universidade de São Paulo, Cx. Postal 97, Piracicaba, SP, BRAZIL, 13416-970; Instituto de Química, Depto. Química Analítica, UNICAMP, Campinas, SP, BRAZIL.

Different factors can be related to morphogenesis *in vitro*, such as explant physiological condition, culture medium composition, biochemical parameters, among others. Culture conditions, carbon source and mineral nutrients, such as Ca, Mg and K, can play important roles in the morphogenic expression, by triggering or participating in different biochemical processes. Embryogenic callus cultures of citrus respond to cabon sources with the development of numerous somatic embryos. This response, however, is variety and carbon-source dependent, making it a nice system to study parameter changes in embryogenic and non-embryogenic cultures. The present study aimed to characterize some mineral elements (Ca, Mg and K) in callus cultures of two citrus varieties, one considered embryogenic (Valencia) and the other non-embryogenic (Rangpur lime) which were subjected to different carbon sources (maltose and sucrose) and temperatures (20, 26 or 32 C), for 30 days. Ca, K and Mg in culture media supplemented with sucrose and maltose were also evaluated. Analysis of proteins, endogenous hormones and carbohydrates are in progress. Three hundred milligrams of callus were subjected to an ultrasonic treatment followed by flame atomic absorption spectrometry. Embryogenic and non-embryogenic callus samples were collected (n 3) weekly and analyzed in triplicate. The results showed that embryogenic calli cultivated in medium containing maltose had higher concentrations of the nutrients compared to non-embryogenic callus cultivated either in sucrose or maltose. Fluctuations in the nutrient levels were observed during the 30-day period of culture under different temperatures. For both varieties a higher decrease in nutrient concentrations was observed from 7 to 15 culture days. After this period only slight differences in these concentrations were achieved. However, these fluctuations were more evident for Ca and K in the embryogenic calluses, indicating a possible participation of these elements in the embryogenic process. (Financial support FAPESP, CNPq)

P-1025

Using Tissue Culture to Generate *Phragmites*-blocking Wetland Plants. J. WANG, J.L. Gallagher, and D.M. Seliskar. Halophyte Biotechnology Center, College of Marine Studies, University of Delaware, Lewes, DE 19958. E-mail: wangjb@udel.edu

Phragmites australis, or common reed, overtakes thousands of acres of wetlands in the United States every year, out-competing more beneficial plants that are desirable for maintaining the balance of the wetland ecosystem. Currently, the primary way of controlling *Phragmites* is two consecutive years of spraying the plants with a herbicide in fall and burning the dead canes in spring. A vegetation alternative to the possible need to respray an area relies on the development of varieties of desired species that can block the reinvasion of *Phragmites* from where it has been eliminated and subsequently planting these varieties at the key points where *Phragmites* can reinvade and spread. We are tissue culturing several species of potential "Phrag-blockers" and using somaclonal variation selection to enhance characteristics, such as dense root systems and thick upright shading canopies, and possibly allelopathic properties. Plant regeneration has been achieved in three of the potential Phrag- blockers, *Scirpus robustus*, *Juncus roemerianus*, and *Rumex crispus*. Callus of *Scirpus robustus* was induced from mesocotyls of germinated seedlings when placed on MS medium supplemented with 0.5 mg/L IAA and 0.5 mg/L 2, 4-D. Whole plant regeneration occurred after transferring the callus to MS medium with 0.1 mg/L BA. Juncus roemerianus callus was induced directly from seeds on MS medium containing 1 mg/L NAA, 0.5 mg/L 2, 4-D, 0.5 mg/L BA, and 5 % coconut water. Shoot regeneration occurred on MS medium containing 3 mg/L TDZ . Root induction was achieved after transferring the shoot onto MS medium with 0.1 mg/L NAA. *Rumex crispus* callus was induced from seeds on MS medium supplemented with 0.5 mg/L BA, 0.5 mg/L 2, 4-D, and 1 mg/L IAA. When callus was placed on MS medium containing 0.5 mg/L BA and 0.1 mg/L NAA, shoots regenerated. Root induction was achieved on non-supplemented MS medium.

P-1026

Ethylenediurea (EDU) and the Desiccation Effects of High Concentrations of Ozone on the Jade Plant (Crassula argentea). C. E. BRODERICK, SR. and G. A. Jones, III. Department of Agriculture and Natural Resources, Delaware State University, Dover, DE 19901. E-mail: cbroderi@dsc.edu

The jade plant was selected as a test plant because of its healthy, green, waxy, and shiny, appearance. Although few pathogenic diseases affect jades, ozone (O_3) is one atmospheric gas that produces negative effects on many plants. Its effects on most plants, however, have not been completely described. O_3 is a very reactive species of oxygen that is produced when volatile organic compounds (VOCs) and nitrogen oxides (NO_x) combine in sunlight. Ethylenediurea (EDU), however, is one compound that has been reported to counteract the effects of ozone on plants. This study was conducted to determine visual, physical, and metabolic effects of ozone on jade plants. Cleaned same-sized rooted jade plants were inserted in small glass tubes filled with distilled water or Ethylenediurea (EDU) solution. Tubes, at shoot-root interface, were sealed with parafilm and hung by thread in 1000 ml Erlenmeyer glass flasks. Pipettes funneled gas in and out of the flasks through two-hole stoppers. A Coral Life Aquarium Supply Company ozonizer supplied O_3 at 2 mg/hr and lower concentrations in the continuous flow test apparatus. Experiments were with two treatments and an ambient air control. The wilting shoot apex was the first evidence of O_3 damage after two to three hours. Within twenty-four hours, damage to the shoot apex was clear, with wrinkling of the upper leaves of the plant. Within 72 hours, the apex and leaves died back progressively from the shoot apex to the base of the plant. The ozone-treated plants were desiccated, but those in EDU solutions were less dehydrated. Shoots in distilled water and EDU solution lost some 81.6 and 75.2 percent, respectively, of the total mass in 72 hours. Roots lost 17.6 and 12.8 percent of their masses, respectively. Although the concentrations of O_3 used in the trials were higher that ambient levels, reduction of ozone treatment levels to near ambient levels had similar effects.

VT-1000

The Effects of Different Plant Protein Hydrolysate on Sp2/0 Cells Expressing Recombinant Pro-urokinase. M. C. BORYS, K. D. Hughes, and J. M. Ryan. Biological Development, Abbott Laboratories, North Chicago, IL 60064. Email: Michael.Borys@abbott.com

Hydrolysates from different protein sources such as soy, wheat and yeast have been added to many serum-free or protein-free mammalian cell culture media to improve cell performance. We have investigated the effects of various plant hydrolysates on the productivity of genetically engineered Sp2/0 cells producing recombinant prourokinase. The results showed the addition of soy hydrolysate (Hy-Soy) at 2 gm/liter increased recombinant protein productivity by approximately 10% in serum-free medium containing bovine serum albumin (BSA). A similar increase in prourokinase formation was demonstrated using meat hydrolysate (Primatone) at 2 gm/ liter. The effect of hydrolysate addition on prourokinase formation was further enhanced in medium without BSA. Data will be presented comparing wheat, soy, and yeast hydrolysates with differing degrees of hydrolysis (i.e., percent free amino acids).

VT-1001

A Method for the Synthesis of Stromal Extracellular Matrix (ECM) Synthesized by Normal Human Prostate Cells in Culture. ELIZABETH SCOTTO-LAVINO, Heather L. Sawka, Sanford R. Simon, and Elizabeth J. Roemer, Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794-8691. E-mail: escottol@ic.sunysb.edu

We have developed a method for in vitro synthesis of extracellular matrix (ECM) by normal human prostate cells. By adapting culture protocols from our R22 rat heart smooth muscle cells system we have established appropriate parameters for this new model based on commercially available normal human prostate stromal cells. These cells grow very well in either a proprietary serum-free culture medium or similar medium containing 5% FBS (Clonetics). With the addition of ascorbic acid, they will synthesize a stable, fibrous ECM. Culture medium containing either [^{3}H] proline and [^{35}S] sulfate or [[^{3}H] fucose and [^{35}S]-cysteine/methionine is used to selectively radiolabel ECM components. Once the ECM has grown the cells are removed and the matrix composition is analyzed by sequential enzymatic degradation. Individual enzymes: heparinase I & III, trypsin, chondroitinase, collagenase and human leukocyte elastase; are each incubated with the ECM for twenty-four hours, to extract individual matrix components. Supernatants from each step of the sequence are read by scintillation counting and analyzed to determine the relative proportions of components in the original, intact ECM. Enzyme-free control wells for each sample are incubated with the appropriate buffers and analyzed for spontaneous degradation of matrix, providing a relative measure of the ECM's physical stability and strength. Thus far, we have examined prostate stromal cells of 3 different ages: a 17, a 37 and a 42 year old (17y, 37y, 42y). Both total count analysis and sequential digestion reveal that the ECM of all 3 cell lineages incorporate all 4 radiolabels: fucose, methionine/cysteine, proline, and to a lesser extent sulfate. Total proline incorporation at day 7 is approximately double to that at day 4 in the ECM of both HPS 17y and 42y. We will continue to develop this in vitro human matrix system for use both as part of a co-culture system with normal prostate epithelium and as a substrate for study of the degradative behavior of human prostate tumor cells. This study was supported by NIH(NIDCR) DE-10985; CollaGenex Pharmaceuticals, Inc.; USAMRMC DA-MD-1798-18560; SUSB Center for Biotechnology & URECA #264900.

VT-1002

Conditional Immortalization of Human Prostate Epithelial and Mesenchymal Cells. J.R.W MASTERS, M.J. O'Hare, B. Daly-Burns, and D.L. Hudson. Institute of Urology, University College London, 3rd Floor, 67 Riding House Street, London W1W 7EY, UK. E-mail: J.Masters@ucl.ac.uk

The aim of our study is to develop in vitro models of human prostate cells that can both grow and differentiate. We have used a temperature-sensitive (conditional) mutant of the SV40 large T-antigen (tsA58-U19) to extend the growth of both epithelial and mesenchymal cells from biopsies of benign prostatic hyperplasia. Following collagenase digestion, primary epithelial cultures were developed in serum-free medium (BioWhittaker) and mesenchymal cultures in standard medium (RPMI-1640/10% FCS) and transduced with the tsT gene using an amphotropic retroviral vector. Following selection in G418, the transduced cells were maintained at the permissive temperature of 33.5 C. When the temperature is switched to 39 C, the rate of cell division falls in both the epithelial and mesenchymal cells due to a conformational change inactivating the tsT gene, thus allowing the cells to grow and differentiate under normal cell control mechanisms. At the permissive temperature, the majority of epithelial cells express cytokeratin 14 and at the non-permissive temperature the proportion of epithelial cells expressing 8/18 increases, indicating differentiation towards a luminal phenotype. Most of the mesenchymal cells express smooth muscle alpha -actin. Combinations of conditionally immortalized prostate epithelial and mesenchymal cells grown in Matrigel produce three-dimensional structures with a basal and luminal layer. These cell systems constitute a step towards a functional in vitro model of the human prostate.

VT-1003

Population Dynamics of Spheroid Self-Assembly of Prostate Cancer Cells. R.M. Enmon, K.C. O'CONNOR, D.J. Lacks, D.K. Schwartz, and R.S. Dotson. Tulane Cancer Center, Department of Chemical Engineering and Department of Surgery, Tulane University and Medical School, New Orleans, LA 70118. Email: KOC@TULANE.EDU

Multicellular spheroids generated by in vitro self-assembly of cancer cells resemble micrometastases and avascular regions of larger tumors especially from the perspectives of differentiated function and spatial organization. Their applications include in vitro drug testing and basic research where frequently a significant portion of the spheroid population is often excluded to produce a more uniform size distribution. In contrast, we demonstrate that significant insight can be obtained into culture properties by considering the dynamics by which the entire cell population self-assembles into spheroids. To this end, we investigated the dependence of intercellular adhesion and cell motility on spheroid size and incorporated this information into a population balance, which predicts the spheroid size distribution throughout cultivation. This research employed multicellular spheroids of DU 145 human prostate cancer cells formed in liquid-overlay culture over a 24-hr period as a model system. With time-lapse video microscopy, the adhesion probability between spheroids was found to increase on average from 16.6% for cell-cell collisions to 45% for spheroid-spheroid collisions and to 83% for cell-spheroid collisions. This was accompanied by a rapid decline in cell motility by a factor approaching ten when single cells dimerized, a far greater reduction than predicted by the change in mass alone. With in situ ELISA, we analyzed the accumulation of a representative cell adhesion molecule, E-cadherin, and matrix protein, collagen type IV, on the spheroid surface to explore the biological mechanisms underlying these physical changes. E-cadherin is involved directly in intercellular adhesion; collagen IV, indirectly through integrin binding. For both biomarkers, the staining intensity per positive cell was unchanged by spheroid formation; however, the percentage of positive cells dramatically increased on average from 27% to 91% for collagen IV and 18% to 100% for E-cadherin. These findings suggest that DU 145 cells were activated upon spheroid formation to become more adhesive through an up-regulation of adhesion molecules and matrix proteins in non-expressing cells. In so doing, cell-cell interactions became favored over cell-substrate interactions, resulting in lower motility. Time-dependent changes in the spheroid size distribution can be predicted with a population balance in which adhesion becomes more probable with larger spheroids and cellular motility remains size-independent, indicating that adhesion, and not motility, governed the rate of spheroid self-assembly in our system. We envision that the methodology presented here will be useful in assessing the adhesive properties of tumor cells as a measure of metastatic potential and in evaluating new drugs that seek to alter intercellular adhesion.

VT-1004

Activin A Promotes Differentiation of the Salivary Gland Stem Cells into the Acinar Cells. M. FURUE[1,3], Y. Zhang[2], T. Okamoto[2], R-I. Hata[1], and M. Asashima[3,4]. [1]Department of Biochemistry and Molecular Biology, Kanagawa Dental College, Yokosuka, 238-8580, JAPAN; [2]Department of Molecular Oral medicine and Maxillofacial Surgery I, Hiroshima University School of Dentistry, [3]Department of Life Sciences (Biology) and [4]CREST Project, University of Tokyo, JAPAN. E-mail: mihofuru@kdcnet.ac.jp

We have previously established a rat submandibular gland (SMG)-derived epithelial cell line (RSMG-1) to study the mechanism of morphogenesis in salivary gland development and regeneration. RSMG-1 cells have biological characters of the salivary gland stem cells. We found that activin A regulated the branching morphogenesis of RSMG-1 cells, suggesting that it is involved in SMG morphogenesis. We used a subtraction cloning procedure with activin-A-treated and untreated RSMG-1 cells to identify activin-A-induced genes. One of the genes detected encoded a rat homologue of *Sel-1l* (*rSel-1l*). Northern blot analysis revealed that activin A induces *rSel-1l* mRNA expression in RSMG-1 cells, and *in situ*hybiridization revealed that *rSel-1l* is intensely expressed in SMG acinar cells and RSMG-1 cells cultured with activin A. The SMG intercalated duct (ID) cells express low level of *rSel-1l* or not at all in vivo. These results suggest that activin A promotes differentiation of RSMG-1 cells and the ID cells into acinar cells.

VT-1005

Autonomous and Human Papillomavirus Enhanced Replication of Adeno-associated Virus Type 2 in an In Vitro Organotypic Culture System. S. Alam*, P.L. Hermonat#, M. Mane#, and C. MEYERS*. *Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033; and #Department of Obstetrics and Gynecology, University of Arkansas for Medical Sciences, Little Rock, AR 72205. E-mail: cmm10@psu.edu

Adeno-associated virus type 2 (AAV2) has been classified as a helper-dependent parvovirus. Recently, using an organotypic (raft) epithelial culture system capable of complete stratification and differentiation, we demonstrated the complete autonomous AAV2 life cycle. AAV2 vegetative replication was directly correlated with epithelial differentiation and non-differentiating keratinocytes were deficient for AAV2 replication. Sero-epidemiological and laboratory studies suggest that AAV may have an inhibitory effect against the development of human papillomavirus (HPV) associated cancer. We therefore analyzed the affect of AAV2 superinfection on HPV productively infected host tissues. Our organotypic culture system is also capable of supporting the complete life cycle of HPV in vitro. HPV-infected organotypic culture tissues were superinfected with AAV2. We observed a multiplicity of infection- (MOI-) dependent enhancement and inhibition of HPV DNA replication, concomitant with AAV2 replication. Specifically, at low MOIs of AAV2 infection, HPV DNA replication was increased compared to controls and AAV2 replicated to high levels. AAV2 replication was greatly augmented in the presence of HPV compared to primary keratinocyte, HPV-negative squamous cell carcinoma, and HPV-negative HaCat organotypic tissue cultures infected with AAV2 alone. Therefore, HPV provided types of "helper/enhancer" functions for AAV2 replication and progeny formation. Infection with AAV2 had significant effects on epithelial morphology. Our results demonstrate a complex interaction between AAV2 and HPV in natural host tissue.

VT-1006

13-*cis*-Retinoic Acid Up-Regulates Surface Expression of CD40 on Human Dendritic Cells During their Differentiation *In Vitro*. M CHIRIVA-INTERNATI[1], F. Grizzi[2], C. Carter[3], P. Hermonat[3], and N. Dioguardi[2]. [1]Center for Immunology and Microbial Disease, Albany Medical College, Albany, NY; [2]Scientific Direction, Istituto Clinico Humanitas, Rozzano, Milan, ITALY. [3]Division of Gynecologic Oncology, University of Arkansas. E-mail: chirivm@mail.amc.edu

High expression of MHC antigens and adhesion/costimulation molecules is considered as one of the major characteristic qualifying human dendritic cells (DCs). Retinoids are potent regulators of cell growth and differentiation. In this study we investigated the effect of *13-cis*-retinoic acid (RA) and *all-trans*-RA on the kinetics of expression of MHC antigens and several adhesion/costimulation molecules of human DCs during their differentiation *in vitro*. PBMC were isolated from peripheral blood of ten myeloma volunteers. These cells were then plated ($1x10^7$/3 ml per well) in AIM-V culture medium. After 2 h at 37 ° C, non-adherent cells were removed, and the adherent cells were further in medium supplemented with recombinant human GM-CSF (800 U/ml), IL-4 (1000 U/ml) to stimulate differentiation into DCs. Some of these adherent cells were treated with *13-cis*-RA and *all-trans*-RA at the same concentration (1iM, Sigma, St. Louis, MO) for 10 days. Finally the cell cultures were treated with TNF-á and IL-1 on days 7-10 to stimulate complete DCs differentiation. At various times (days 1, 2, 5, 7 and 10) the cultures were evaluated for surface markers expression using FACS analysis. For each time point, a panel of mAbs recognizing the following antigens was used: CD40, CD54, CD80, CD1a, CD86, CD14, and CD83. It was found that significantly higher expression of CD80, CD86, and CD54 occurred in the retinoids treated DCs cultures (60%-80% increase), while CD1a was unaffected. However, the most prominent difference was for the expression of CD40, which was over-expressed by 90%-100% in the presence of *13-cis*-RA. CD40 expression has been shown to be crucial not only for B cell growth, isotype switching, and Ig synthesis, but also for optimal T cell priming. Our results suggest that retinoids can alter important surface marker levels on DCs; moreover, this study supports the possibility that *13-cis*-RA may be a useful agent for improving cancer immunotherapy.

JP-2000

In Vitro Propagation and Quantification of Rotenoids in Callus of Derris sp. J.E.B.P. PINTO; H.E.O. Conceição; N.E.A. Castro; E.J.A. Santiago, and O.A. Lameira. Laboratory Tissue Culture, UFLA Cx.P.37, LAVRAS-MG, 37200-000 BRAZIL. E-mail: jeduardo@ufla.br

The Amazonian ecosystems are rich in plants with insecticide properties. In this work, in vitro techniques of propagation and quantification or rotenoids in callus of Derris sp were applied. In vitro propagation of na endangered insecticide plant was achieved by culturing the nodal segment explant. Nodal segment containing two axilar buds showed better development in number and size of the shoots than one axilar bud. Shoots were rooted on MS/2 basal medium and soaked for 30 seconds in indole-3-butyric acid (IBA) at 2,000 mg/L with pH adjusted to 4.5. Derris sp did not show any multiple shoots type. This species showed multiplication through nodal segments. Plantlets with a morphologically normal appearance were transferred to soil and acclimated in the growth chamber for 30 days. Callus culture were established from root segment of seedlings germinated in vitro on Murashige and Skoog (MS) basal medium supplemented with 1.6 mg/L naphthaleneacetic acid (NNA) + 1.0 mg/L benzylaminopurine (BAP). The most efficient culture medium of maintanance of callus was provided on basal MS medium supplemented with 2.0 mg/L NNA + 2.0 mg/L BAP. Callus from root segment presented positive response to biosynthesis of rotenoid compound.

JP-2001

Extraction and Detection of Kavapyrones from In Vitro Cultures of Kava (*Piper methysticum* Foster). H. Kobayashi, M.A.L. Smith, M. Gawienowski, and D. Briskin. Dept. of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801. E-mail: hkobayas@uiuc.edu

The roots and rhizomes of kava (*Piper methysticum* Foster), a South Pacific medicinal herb, are used as a phytomedicinal treatment for anxiety, tension, agitation, and insomnia. Slow maturity, sterility, and diseases threaten the supply of this medicinal herb. The objectives of this study are to develop kava micropropagation and kavapyrone production *in vitro* to support conventional kava production and future bioreactor-based production of kava phytomedicinals. Young, expanding leaves from greenhouse kava plants ('Awa' and 'Makea') were introduced to modified 1/2 Murashige and Skoog media containing Plant Preservative Mixture (PPM, 2.0 ml L^{-1}), and, in mg L^{-1}, 2,4-dichlorophenoxyacetic acid (2,4-D, 2.0), or a-naphthalenacetic acid (NAA, 0.1) and N6-benzylaminopurine (BA, 0.5). Despite severe and persistent contamination, callus initiation subsequently occurred on media with 2,4-D after four weeks, and formation of protuberances resembling embryos were observed within two months. Root regeneration occurred after transfer of calli to within one month to 1/2 MS media with NAA at 2.0 mg L^{-1}. High Performance Liquid Chromatography and Thin Layer Chromatography analyzed the methanolic extraction of callus and regenerated roots from callus, along with roots of greenhouse plants. The amount of kavapyrones detected from the callus sample by HPLC was significantly less than that of kava roots from the greenhouse, while the amount of kawain from regenerated roots was comparable to that of roots *in vivo* on the TLC plate.

JP-2002

Light Does Not Regulate All Steps in the Mevalonate Independent Pathway of Terpenoid Biosynthesis. F. SOURET, P. Weathers, K. Wobbe. Departments of Biology and Biotechnology, and Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, MA 01609. E-mail: souret@wpi.edu

Two distinct terpenoid pathways have been characterized in higher plants leading to the biosynthesis of isopentenyl diphosphate: the well-described cytosolic mevalonate pathway and the recently characterized mevalonate independent pathway, postulated to be located in plastids. It was recently demonstrated that the mevalonate independent pathway could also be involved in sesquiterpene production that normally occurs in the cytosol. *Artemisia annua* transformed hairy roots produce artemisinin, a sesquiterpene lactone, with effective anti-malarial activity. Considering the importance of sesquiterpenes as natural products and using transformed roots of *A. annua* as a unique biotechnological model to study the regulation of terpenoid biosynthesis, we decided to investigate the key enzymes involved in the mevalonate independent pathway. Of particular interest is the enzyme performing the first committed step in the mevalonate independent pathway, 1-deoxyxylulose-5-phosphate reductoisomerase (DXPR). Using RT-PCR, we have isolated a partial DXPR cDNA that was then used to screen a cDNA library. We isolated a 2.2 kb putative cDNA clone characterized by a 1.4 kb ORF encoding a mature protein of 471 aa. Bacterial expression of DXPR cDNA confirmed that the cDNA encodes a protein of roughly 50 kDa. Northern blot analysis showed that DXPR was constitutively expressed in normal greenhouse-grown plants and in transformed roots and was not affected by culture age. Moreover, while light exposure caused a significant transcriptional upregulation of DXPS, the enzyme immediately upstream of DXPR, the relative transcription levels of DXPR was unchanged upon light exposure. We are currently investigating other factors that could influence DXPR mRNA levels in our transformed roots.

JT-2003

In Vitro Effects of Semipure Protease Inhibitor Fractions from Edible Seeds on Malignant Cell Survival. T. GARCIA-GASCA, L. A. Salazar-Olivo, E. Mendiola-Olaya, C. Aguirre and A. Blanco-Labra. Department of Biochemistry and Biotechnology, Cinvestav Unit for Biotechnology and Genetic Engineering, Chemistry School and Medicine School, Queretaro Autonomous University. P. O. Box 184, Queretaro 76010, Qro. MEXICO. E-mail: alter@sunserver.uaq.mx

Protease inhibitors (PI) have been described as the first diet component with anticancerigen potential. Among them, the one that has received more attention is the soybean Bowman-Birk inhibitor, which presently is under clinical trials to assess its suitability as a therapeutic agent. However, few PI form different sources have so far been investigated on this context. Here, we report on the effect of two semipure PI fractions extracted from seeds of chan (*Hyptis suaveolens*) (C-PIF) and amaranth (*Amaranthus hypochondriacus*) (A-PIF), on the survival of transformed cells. Protein fractions with PI activity, obtained after purification of a protein extract of those two seeds through a G-75 Sephadex column, were tested *in vitro* using three different cell lines: HeLa cells, a human transformed cell line from epithelial origin; a murine transformed fibroblastic cell line, NIH 3T3/Ha-ras; and a normal fibroblastic 3T3 cell line. Normal and transformed fibroblasts were maintained in Dulbecco's modified Eagle medium (DMEM) with 5% calf serum (CS), whereas for HeLa cells, the same conditions were used but 5 μg/mL insulin were added; all cell lines were incubated at 37° C under a 10% CO_2-90% air humidified atmosphere. Cells were seeded (0.8 or 1.0 x 10^4 cells /well) in 24-multiwell plates with 5% CS DMEM. After 48 h, medium was removed and cultures were treated with 100, 500, 750 and 1000 UI/mL of C-PIF or A-PIF in DMEM containing 1% bovime serum albumine (ASB). Two control treatments were included: DMEM supplemented with 1% BSA and DMEM added with 5% CS, respectively. After 72 h, cell number was estimated. Dose-response curves showed that survival of HeLa cells was not affected by C-PIF, however A-PIF decreased cell proliferation up to 26%. Proliferation of transfromed 3T3/Ha-ras fibroblasts was decreased up to 59% and 49% in the presence of C-PIF and A-PIF respectively. Contrarily, normal 3T3 fibroblasts were not affected by any of the two treatments. These results show that both, C-PIF and A-PIF affected cell survival of only the transformed cells, and that such effect is dependent on cell lineage and transformation level.

JT-2004

The Antioxidation Effects of Fermented Products and Food Extracts in CHO-K1 Cells. JIAN-CHYI CHEN, Yu-Hui Wei, Rong-Zhong Xie, Shu-Wan Wang, and Shan-Shan Chen. Culture Collection and Research Center, Food Industry Research & Development Institute, P.O. BOX 246, Hsinchu 300, TAIWAN. E-mail: JCC@FIRDI.ORG.TW

The protection effect of fermented products (anka and glutathione) and food extracts (catechin, lipid and soybean sauce meal), against hydrogen peroxide-induced cytotoxicity, was investigated in a Chinese hamster ovary cell line (CHO-K1) during this experiment. In order to evaluate the antioxidative effects of these products, cells were treated with two different ways: 1) Cells were co-incubated with H2O2 and product; 2) Before treated with H2O2, cells were pre-treated with product. The cytotoxicity effects of H2O2 on cell growth were determined by using the tetrazolium dye colorimetric test (MTT test). The results have showed that the treatment of CHO-K1 cells with 0.04 mM H2O2 reduced their viability 80% related to the control group. Pre-treatment of cells with 9.6 ug/ml soybean sauce meal, 250 ng/ml lipid, 200 ug/ml anka, or 16.8 ug/ml catechin for 24 hr could increase cells viability for about 13%, 16%, 23% or 27%, respectively. In addition, co-incubated cells with H2O2 and glutathione, the viability evenly reached 90%. Our results suggest that glutathione plays a role of H2O2 scavenger. However, the real mechanisms of other products on protection of cellular cytotoxicity remain unknown. So, further studies are necessary to find out the exact mechanism of our products.

I-2000

Scanning Electron Microscopy of Midgut Epithelial Cells from *Dendroctonus valens* (Coleoptera:Scolytidae) Maintained *In Vitro*. L. SANCHEZ, J.L. Andrade, Ma.E. Sánchez, R. Cisneros, and G. Zúñiga. Laboratorio de Variación Biológica y Evolución. Departamento de Zoología, Escuela Nacional de Ciencias Biológicas-IPN. MEXICO, D.F. 11340. E-mail: lchapul@yahoo.com

One of the most critical steps in the establishment of culture conditions to maintain midgut epithelial cells *in vitro* has been cell adherence. It has been reported that attachment to a substratum increases the growth of epithelial cells. The purpose of this study is to promote cell attachment to a glass surface and to make more detailed observations of their morphology by scanning electron microscopy (SEM). Midgut epithelial cells from *D.valens* were grown between two glass coverslips (like sandwich) placed at the bottom of each well in a 24-well multidish plate. Culture was incubated for 30 days at 28°C (microaerofilic atmosphere) in RPMI 1640 medium supplemented with 10% fetal calf serum, 20-hydroxyecdysone and fat body extract from *Manduca sexta*. The cells attached to the glass surface were fixed with 2.5% glutaraldehyde in PBS for 2 h at 4°C and subsequently postfixed with 1% osmium tetroxide for 2 h at room temperature. After that samples were dehydrated with ethanol and critical-point dried. The samples were coated with gold-palladium and analized by SEM. Light microscopy observations in the 15th day show that the cells adhere and spread out on the surface of the coverslip forming a thin and small adherent sheet through long cell projections. The adherent cell surface observed by SEM is wrinkled, convoluted and porous. They also emit short cytoplasmic prolongations that led the cells adhere to the glass surface. Scanning electron micrographs of intact tissue cells confirmed the surface characteristics of cultured cells. The presence of the coverslip pressing down on the cells seems to be an important factor that promotes cell attachment and morphological changes because cells now appear like fibroblast instead of epithelial cells *in vitro*.

P-2000

Somatic Hybrids of *Solanum tuberosum* cv. Desiree and *S. chacoense* Bitt: A Baseline for Disease Resistance in Potato. B. SADIA, P. Anthony, J.B. Power, K.C. Lowe, and M.R. Davey. Plant Science Division, School of Biosciences, University of Nottingham, Nottingham NG7 2RD, UK. E-mail: mike.davey@nottingham.ac.uk

Solanum chacoense, a wild tuber-bearing species (2*nx* 4), is resistant to potato cyst nematode, colorado beetle, common scab and bacterial wilt. It contains glycoalkaloids (leptines) associated with insect/disease resistance. In attempts to introduce these characteristics into tetraploid (2*nx* 8) cultivated potato (*S. tuberosum* cv. Desiree), mesophyll protoplasts of *S. chacoense* were electrofused with cell suspension protoplasts of potato, giving 12% heterokaryon formation. Cultured protoplasts of *S. chacoense*, did not produce colonies. However, in the same medium (MS with 1.25 mgl^{-1} NAA, 0.25 mgl^{-1} 2,4-D, 1.0 mgl^{-1} zeatin or 0.1 mgl^{-1} 2,4-D, 0.3 mgl^{-1} BAP), protoplasts of Desiree were totipotent. Selection of putative somatic hybrid tissues was based on heterosis, with such tissues exhibiting both purple and green pigmentation. Two hundred calli were obtained from 4 experiments; 75 vigorously growing putative hybrid calli were selected and transferred to regeneration medium (MS with 0.02 mgl^{-1} NAA, 0.02 mgl^{-1} GA_3, 2.0 mgl^{-1} zeatin). After 16 weeks, tissues regenerated 24 plants with anthocyanin pigmented stems, a characteristic of *S. chacoense*. RAPD analyses indicated the hybridity of 9 plants after transfer to the glasshouse. Three of twenty four 10-mer primers tested showed the presence of parental DNA bands in these plants. The latter were intermediate in their vegetative, floral and tuber characters compared to both *S. chacoense* and *S. tuberosum*. After 4 months in the glasshouse, plants produced larger, red tubers similar to those of Desiree, compared to small white tubers of *S. chacoense*. As expected, regenerated plants were amphidiploids, with a chromosome complement of 2*nx* 2. Somatic hybrid plants are being evaluated for their disease resistance.

P-2001

Introduction of Sweetpotato Feathery Mottle Virus-Coat Protein Gene into US and South African Sweetpotato Varieties via Agrobacterium tumefaciens. C. L. DANIELS, M. Egnin, and C. S. Prakash. Center for Plant Biotechnology Research, CAENS, Tuskegee University, Tuskegee, AL 36088. E-mail: chantald1@hotmail.com, megnin@tusk.edu

Annually, more than 60-100% of sweetpotato yield is lost due to abiotic stresses and biotic factors such as insect pests, nematodes, and viral diseases. Potyviruses, primarily sweetpotato feathery mottle virus, are amongst the most destructive agents of sweetpotato in Africa. Developing resistance to this virus through gene transfer is a logical approach because of a lack of such resistance in sweetpotato germplasm. The SPFMV-coat protein (*spfmv-cp*) gene was introduced, via *Agrobacterium* system into one US and several South African (SA) sweetpotato varieties. An initial study showed that kanamycin selection of embryogenic explants beyond 20mg/l to be lethal, while selection at 10mg/l allowed explants to maintain their embryogenic potential and regenerate shoots. To eliminate the potential for "escapes" during regeneration, kanamycin selection at 15mg/l was chosen for further transformation studies. *Agrobacterium* strains, C58 and EHA101, each harboring the binary vector, pGCN1559, containing the *nptII* gene, and the *spfmv*-coat protein (sense) or the antisense *vmf* genes driven by CaMV-35S promoters, were used to transform leaf explants. Cocultivated explants were cultured on callus production media with 2,4-D (1.0mg/l) and BAP (0.25mg/l) for 5 days before being transferred and continuously selected on various regeneration media with kanamycin (15mg/l). Putative transgenic shoots were recovered from transformed explants for both the sense and antisense coat protein genes. A total of 62 regenerants are being tested for the integration and expression of the coat protein gene. *Research supported by USDA-ARS and NASA.*

P-2002

Regenerants Derived from Leaf Explants of Several Strawberry Cultivars, Exhibit Increased Levels of Resistance to the Fungal Pathogen *Colletotrichum acutatum*. F.A. HAMMERSCHLAG[1], S. Garces[1], M. Koch-Dean[2], J. Maas[1], and B. Smith[3]. [1]USDA/ARS, Fruit Laboratory, BARC-West, Bldg. 010A, Beltsville, MD 20705; [2]ARO, The Volcani Center, Institute of Field and Garden Crops, Bet Dagan, ISRAEL 50250; [3]USDA/ARS, Small Fruit Research Station, Poplarville, MS 39470. E-mail: HAMMERSF@BA.ARS.USDA.GOV

Regenerants, from leaf explants of strawberry (*Fragaria* x *ananassa*) cultivars Chandler, Delmarvel, Honeoye, Latestar, Pelican and Sweet Charlie were generated on MS medium containing either 1 or 10 microM thidiazuron and 0.49 microM indole-3-butyric acid (IBA), propagated in vitro on medium containing MS salts, 4.4 microM 6-benzyladenine and 5.7 microM IBA, and 4 wk prior to screening, were transferred to propagation medium without growth regulators. Regenerants and cultivars were screened in vitro by soaking leaves from 4-wk-old cultures in a spore suspension of the pathogen *Colletotrichum acutatum* isolate Goff (causal agent of anthracnose) for 24 h, subculturing leaves onto 0.5 % Difco Bacto agar, and then scoring leaves for percentage infection after 7 d. Regenerants exhibited 3.5-, 1.7-, 1.7-, 2.1-, 1.4- and 3.9-fold increases in levels of disease resistance compared to cultivars Chandler, Delmarvel, Honeoye, Latestar, Pelican and Sweet Charlie, respectively. Maximum levels of resistance to *C. acutatum* were exhibited by regenerants CS-1 and CS-10 (from 'Chandler'), and SS-3, SS-8 and SS-9 (from 'Sweet Charlie'). CS-1, CS-10, SS-3, SS-8 and SS-9 exhibited 17.5, 17.7, 11.7 14.9 and 13.9% leaf infection, respectively, compared to 62.4 and 45.1% for 'Chandler and 'Sweet Charlie', respectively. These studies suggest differences in genetic stability among strawberry cultivars cultured in vitro, and that screening for somaclonal variation may be a feasible approach to increasing levels of anthracnose resistance in strawberry cultivars.

P-2003

Constitutive Expression of Scab-inducible Genes for Enhancing Disease Resistance in Wheat. A. ANAND1, W.L. Li2, N. Sakthivel1, S. Krishnaveni1, S. Muthukrishnan1, B.S. Gill2, H.N.Trick2. 1 Department of Biochemistry, 2 Department of Plant Pathology, Kansas State University, Manhattan, KS 66502. E-mail: ajith@ksu.edu

A cDNA library constructed from spikelets of Sumai 3, a scab-resistant cultivar, inoculated with conidia of *Fusarium graminearum* was used to identify genes for novel PR-proteins induced on scab-infection. Two chitinase and two beta-1,3-glucanase clones were isolated using a rice class I chitinase and barley class II chitinase cDNA clone and a barley beta-1,3-glucanase as probes. Northern blot hybridization showed that the expression of these genes is induced upon infection with *Fusarium graminearum*. The spring wheat, 'Bobwhite', a scab-susceptible cultivar was transformed with pAHC20 vectors carrying the *bar* gene and the gene of interest under the control of maize ubiquitin promoter-intron. Twenty-three primary transgenic lines with different gene-combination(s) were identified based on PCR detection for *bar* gene, gene(s) of interest and western blot analyses. The integration and inheritance of the transgene and the *bar* gene were followed in the T_1 progenies based on Southern hybridization with target gene probes and PCR analyses. Liberty (0.1%) painting and western blot analyses confirmed stable expression of the transgene. Several transgenic lines containing single or different combinations of PR-protein genes have been identified and are being propagated, for bioassay.

P-2004

Optimization of Growth and Particle Bombardment-mediated Transformation of Embryogenic Soybean Tissue, Maintained on a Semi-solid Medium. J.J. FINER and A.J. Staron. Department of Horticulture and Crop Science, The Ohio State University. Wooster, OH 44691. E-mail: FINER.1@OSU.EDU

Over the past few years, embryogenic tissue of soybean has emerged as the tissue of choice for particle bombardment-mediated transformation studies. In previous transformation studies, embryogenic suspension cultures were used, as growth of tissue in liquid cultures was apparently more rapid compared with tissue grown on a semi-solid medium. Unfortunately, embryogenic suspension cultures of soybean can be difficult to initiate and maintain, and regenerated plants are prone to sterility problems. We have recently developed transformation systems for soybean using embryogenic soybean tissue (D20 tissue, maintained on a semi-solid medium containing 20 mg/l 2,4-D) with both the particle gun and *Agrobacterium*. As this tissue is slow growing, efforts were made to optimize growth of this tissue by evaluating environmental conditions and media addenda. Maintenance of D20 cultures at 23°C or 25°C, rather than our standard laboratory conditions of 27°C, resulted in both enhanced growth rates and higher tissue quality (as judged by tissue morphology and color). Tissues maintained at 23°C and 25°C also appear to be more responsive to transformation. Addition of asparagine or glutamine to cultures maintained at 25°C also resulted in increased growth rates with an additional increase in transformation competency. However, comparison of transformation competency of tissue grown on media containing various levels asparagine or glutamine indicated that higher growth rates were not always precisely correlated with higher transformation competency. It appears that transformation competency is often correlated with growth rate, but this is not always the case.

P-2005

Elevated Agar Concentration in the Cocultivation Medium Considerably Improves Efficiency of Agrobacterium-mediated Transformation of Tomato. SERGEI F. KRASNYANSKI and Schuyler S. Korban. Department of Natural Resources & Environmental Sciences, University of Illinois, Urbana, IL 61801. E-mail: ksergei@staff.uiuc.edu

Often, inoculation of explants with different *Agrobacterium* strains sharing C58 chromosomal background can induce necrogenesis. Necrosis has detrimental effect on the tissue and is directly responsible for lowered efficiency of T-DNA transfer. To investigate this phenomenon in an attempt to overcome tissue necrosis, *in vitro* grown 8-days old cotyledons of tomato *cv*. Sweet Chelsea were used for transformation. *Agrobacterium* strain GV3101(pMP90) with a binary vector carrying the selectable marker gene *nptII* along with a *uidA*-Intron chimeric gene driven by the 35S CaMV promoter was used for inoculation of cotyledonary explants. Following 48 h of cocultivation with *Agrobacterium* on media containing 6-, 8-, 10-, and 12 g/L agar, explants were cultured on a regeneration medium containing 100 mg/L kanamycin and 500 mg/L carbenicillin. After 3 to 5 days, necrotic areas at wounding sites were observed. Within 10 to 15 days after inoculation, up to 30% of explants cocultivated on media containing 6 or 8 g/L agar were dead due to necrosis of entire tissue. However, only 15 or 5% of cotyledons cocultivated on media containing 10 or 12 g/L agar, respectively, were dead because of necrosis. No necrotic response was observed in control explants (were not inoculated or cocultivated with *Agrobacterium*). A two-fold increase in transformation efficiency (number of GUS positive i.e. transformed shoots per explant) was observed when explants were cocultivated on a medium containing 12 g/L agar *vs.* 6 g/L. These results indicate that presence of a relatively high agar concentration (12 g/L) in the cocultivation medium can increase transformation efficiency of tomato. This may possibly apply to other species displaying necrotic tissue response following inoculation of explants with *Agrobacterium*.

P-2006

Activation of Non-autonomous Maize Transposable Element, *Dissociation (Ds)*, by Ac-transposase in Carrot. A. IPEK and P. W. Simon. USDA-ARS, Department of Horticulture, University of Wisconsin-Madison, 1575 Linden Dr., Madison, WI 53706. E-mail: AIPEK@STUDENTS.WISC.EDU

The mutagenic properties of transposable elements have been explored in many plant species. In order to investigate the possibility of transposon tagging and cloning of carrot genes, carrot was transformed with modified maize transposable elements *Activator (Ac)* and *Ds* using *Agrobacterium tumefaciens* strain LBA 4404. The callus, initially transformed with modified *Ac*, was transformed again with *Ds*. Transgenic carrot plants carrying only *Ds* or both modified *Ac* and *Ds* were analyzed for transposition of *Ds*. *Ds* did not transpose in any of the transgenic plants carrying only *Ds*. On the other hand, *Ds* was excised in all of tissue culture regenerated plants carrying both modified *Ac* and *Ds*. Reinsertion of *Ds* into new chromosomal sites was detected in all of double transformed plants by Southern blotting. Our results indicated that *Ds* will transpose in the carrot genome if Ac-transposase is present. Insertion sites of *Ds* are being sequenced using TAIL-PCR to investigate transposition pattern and secondary transpositions of *Ds*.

P-2007

Regeneration of Transgenics of Picea glauca, P. mariana, and P. abies After Cocultivation of Embryogenic Tissue with Agrobacterium tumefaciens. GERVAIS PELLETIER, Krystyna Klimaszewska, Denis Lachance, and Armand Seguin. Laurentian Forestry Centre, Ste-Foy, Quebec, CANADA. E-mail: gpelletier@exchange.cfl.forestry.ca

Transgenic plants of three Picea species were produced after coculture of embryogenic tissue with the disarmed strain of A. tumefaciens C58/pMP90/pBIV10 and selection on medium with kanamycin. In addition to the nptII selectable gene (conferring resistance to kanamycin) the vector carried the uidA (B-glucuronidase, GUS) marker gene. Transformation frequencies depended on the species, genotype, and post cocultivation procedure. Of the three species tested, P. mariana transformed at the highest frequency, followed by P. glauca, and P. abies. The transgenic state of the embryonic tissue was initially confirmed by colorimetric GUS assay followed by Southern analysis. One to over 5 copies of T-DNA was detected in various transgenic lines analysed. Transgenic plants were regenerated of all species using improved protocols for maturation and germination of somatic embryos.

P-2008

Optimizing the Transformation Efficiency for Flax. K. WARD and M.C. Jordan. Cereal Research Center, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB, CANADA R3C 2M9. Email: kward@em.agr.ca

The flax plant can be transformed using Agrobacterium-based transformation. However, transformation efficiency is low compared to other plant species such as canola. Common selection agents such as kanamycin allow many escapes, causing untransformed plants to be regenerated frequently. In an attempt to improve the transformation efficiency for flax we have constructed Agrobacterium-based vectors which carry three genes between the T-DNA borders - the bar gene which confers resistance to hygromycin, the B-galactosidase gene and our gene of interest we wish to recover in the transformed plant. We have compared three strains of Agrobacterium (LBA4404, EHA105, AGL1) to see if one is superior to the others in its ability to transform flax, as Agrobacterium strains are known to differ in their host range. We have also compared two methods of gene delivery to the flax plant - soaking 1 cm long peeled hypocotyl sections of flax in Agrobacterium culture versus the inoculation of 1-2 mm sections of hypocotyl tissue. The optimum length of the co-cultivation period was also determined. Inoculated tissue was regenerated on hygromycin-containing media. Shoots that regenerate were tested for GUS activity by X-glucuronidase staining of a leaf. By combining both a selectable and a screenable marker gene into the same construct we hope to reduce the number of escape shoots being carried forward, and thus improve the efficiency with which we recover our gene of interest.

P-2009

In Vitro Bioassay of Bt Toxin Expression in a Transgenic Cotton Callus Derived From a Non-regenerable Host Genotype. B. STEINITZ[1], Y. Gafni[1], Y. Cohen[1], S. Levski[2], Y. Tabib[1], and A. Navon[2], [1] Dept of Plant Genetics, Institute of Field and Garden Crops, and [2] Dept. of Entomology, Institute of Plant Protection, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, ISRAEL. E-mail: STEINITZ@NETVISION.NET.IL

Larvae of lepidopterous cotton pests can be bioassayed on callus tissue generated from non-transgenic commercial crop plants. However, the use of callus carrying a transgene encoding for an insect-detrimental substance for such assays has not been addressed in cotton. In the present study we evaluated the insecticidal effects of a Bt toxin in a callus of a commercial *Gossypium hirsutum* L. variety which so far cannot be regenerated into transgenic plants. Transformation of cells was mediated by *Agrobacterium tumefacience*. Two genes were introduced into host cells: the hygromycin B phosphotransferase *hpt* marker gene alone or the *hpt* gene in combination with the Bt *CryIA(c)* toxin gene. Transgenic callus was selected and subcultured on a medium supplemented with hygromycin B. The growth and survival of *Helicoverpa armigera*, *Pectinophora gossypiella* and *Spodoptera littoralis* was examined with neonate larvae reared on three callus types: (i) Non-transgenic. (ii) Transgenic; harboring the *hpt* gene only. (*iii*) Transgenic; carrying the Bt and the hygromycin B resistance gene. We found that the larvae could not grow properly on any transgenic callus tissues harvested from hygromycin-supplemented medium. However, larvae grew normally when fed on non-transgenic callus. This larval growth was supported also on hygromycin-resistant callus that was sub-cultured on a hygromycin-free medium for 3 months before conducting the bioassay. Our results indicate the following: (a) Callus from medium with hygromycin B was detrimental to neonates. (b) When hygromycin B was omitted from the medium, the transgenic callus carrying the *CryIA(c)* toxin gene impeded larval development due to toxicity of the Bt protein. The insect - transgenic callus assay provides a useful tool to study the biological activity of a transgene in cells of a non-regenerable plant.

P-2010

Early Senescence and Change of Sugar Composition Caused by Expression of a Carrot Acid Soluble Invertase in Tobacco (Nicotiana tabacum L.) Y.Y. YAU and P.W. Simon. Dept. of Horticulture, University of Wisconsin-Madison, Madison, WI 53706.

Carbon source partitioning is an important area for plant improvement. Sucrose is the major carbohydrate transported between plant tissues. Sucrose synthase (EC 2.4.1.13), sucrose phosphate synthase (EC 2.4.1.14), and invertase (β-fructofuranosidase) (EC 3.2.1.26) are the three major enzymes involved in sucrose metabolism. Invertases are present in most plant tissues in multiple forms where they cleave sucrose into fructose and glucose. To study the overexpression of a higher eukaryotic invertase in a plant, carrot (*Daucus carota* L.) acid soluble invertase isozyme I was isolated with RT-PCR and cloned into the transformation vector pBI121, and used to transform tobacco leaf discs. Southern and northern hybridization confirmed the existence of the transgene. Two kinds of transgenic plant phenotypes were observed. One appeared normal and showed transgene silencing due to at least two transgene copies. The other had yellowish older leaves and contained only one single copy of the transgene. These symptoms appeared more serious than earlier reports of transgenic plants using yeast invertase. Perhaps this was due to codon usage effect. Leaf sugars from two transgenic plants and one non-transgenic (control) plant were analyzed with HPLC. The HPLC profiles from the transgenic plants were different from the control plant. The growth of T_1 plants was arrested and early senescence of plants was observed.

P-2011

Enhancement of Somatic Embryogenesis by Tryptophan in West African Cassava Cultivars. R.N.N KOKORA, N.J. Taylor, and C.M. Fauquet. International Laboratory for Tropical Agricultural Biotechnology, Donald Danforth Plant Science Center, ILTAB-UMSL CME 308, 8001 Natural Bridge Road, St. Louis, MO 63121. E-mail: nkokora@danforthcenter.org

Cassava (*Manihot esculenta*) is a dominant starchy staple in the diet of African people. Poor seed set and high heterozygosity frustrates conventional breeding and makes genetic engineering an attractive target in cassava. Genetic transformation systems have been developed allowing the introduction of transgenes into embryogenic tissues of the model cassava cv. TMS 60444. However, greater knowledge concerning in vitro factors controlling plant regeneration via somatic embryogenesis is required if genetic engineering technologies are to be transferred to agronomically important cassava cultivars. The established culture system involves the induction of organized embryogenic structures (OES) from leaf explants with subsequent conversion into friable embryogenic callus (FEC); a disorganized tissue of totipotent cells ideal for gene insertion and plant regeneration. The effect of indole-3-acetic acid (IAA) and tryptophan, a natural precursor of endogenous IAA, was investigated in the three West African cultivars, TMS 60444, Bonoua Rouge and Kataoli. Induction of OES was highest in TMS 60444 (69%) when explants were cultured on MS medium supplemented with 50 mM picloram. In Kataoli a maximum response of 56% was obtained while Bonoua Rouge was the least responsive with only 15% of explants undergoing somatic embryogenesis. Tryptophan had no positive effect, while IAA at 50 mM caused a significant reduction in OES induction from the explants. In order to optimize conversion of OES into FEC a range of tryptophan concentrations were added to the culture medium. Inclusion of tryptophan significantly enhanced formation of FEC from OES of cv. TMS 60444 with all concentrations of tryptophan elevating FEC production compared to controls. Optimum response was obtained on Gresshoff and Doy (GD) medium containing 50 mM picloram and 125 mM or 250 mM tryptophan. Cassava plants have been regenerated from tryptophan-induced FEC and are being assessed in the greenhouse. Further experiments are in progress to study the effect of trytophan in other cultivars, while additional IAA precursors will be investigated in order to facilitate the induction of embryogenic tissues and their use as starting material for cassava genetic engineering programs at ILTAB.

P-2012

Field Performance Of Transgenic 'High Protein' Sweetpotatoes (Ipomoea batatas L., PI 318846-3) Show No Yield or Phenotypic Cost of an Extra Gene. M. EGNIN1, C. L. Daniels1, C. S. Prakash1, L. Urban2, T. Zimmerman3, S. Crossman3, and J. Jaynes2. 1. Center for Plant Biotech Research, CAENS, Tuskegee University, Tuskegee, AL; 2. Demegen Inc, Pittsburgh, PA; 3. University of the Virgin Islands, St, Croix. Email: megnin@tusk.edu

The production of transgenic crops expressing novel agronomic traits is a major goal of plant genetic engineering. Transgenic sweetpotatoes, expressing a synthetic storage protein (*asp-1*) gene tailored to provide better human nutrition, were developed at Tuskegee University (TU) and evaluated for performance under field conditions. Five transgenic lines, with enhanced levels of protein and essential amino acids, along with two controls (untransformed, and *gus-nptII* transformed) were field tested in 1997 - 1999 at TU and 2000 in the US Virgin Islands (VI) without or with added nitrogen (80 lb/ acre) using a split plot design. All the transgenic events tested demonstrated protein levels that were two to three folds higher than controls (6.5% to 12% versus 3.1% on a dry weight basis). Transgenic plants did not exhibit any recognizable phenotypic abnormalities, and were similar in traits such as plant height and days to flowering even though they matured later when compared to the controls. Two transgenic lines consistently produced similar or greater storage root yields compared to the control (225 to 296 bushels/acre versus 168 to 188 bushels/acre for 5 plants), and it is not known yet whether the delayed development and relative reduced yields of other lines were a consequence of somaclonal variations or transgene events. All of the transgenic lines showed increased in percent undersized storage roots at TU and VI. Yield was significantly increased under field conditions in VI (33% to 73% over control) than in TU. Results so far suggest that it is possible to select for sweetpotato lines high in total storage protein content and nutritive value with no decline in their productivity by the expression of this *asp-1* gene. If this technology is to have commercial potential it is essential that any yield or other agronomic penalties do not outweigh the benefit of the transgene. *Research supported by NASA and USDA.*

P-2013

Transformation of Ethylene-Response-Sensor(ERS) Mutant Gene in Broccoli(*Brassica oleracea* Var. *italica*) by *Agrobacterium tumefaciens*. L.F.O. CHEN, J.Y. Huang, H.H. Chen, and J.F. Shaw. Institute of Botany, Academia Sinica, Nankang, Taipei, TAIWAN 115. E-mail: OCHENLF@GATE.SINICA.EDU.TW

Ethylene was known to play an important role in the floret yellowing on the post-harvested broccoli. An ERS (ethylene-response-sensor) mutant gene, lacking of receiver domain for ethylene in broccoli has been cloned by Dr. J.F. Shaw's laboratory in our institute. Two plasmids were constructed with this gene regulated by the CaMV 35S promoter together with anti-biotic nptII (kanamycin resistance gene) coding sequence and hph (hygromycin resistance gene) respectively for the pBI-mERS162F and pSM1H-ERS162F plasmids. Genetic transformation of the above two constructions via *A. tumefaciens* has been conducted to evaluate their effects on floret yellowing of harvested broccoli. Presently, several transformants have been obtained through *A. tumefaciens* infection on the selected cotyledon and hypocotyl explants. The pSM1H-mERS162F was found to have a better transformation rate than that of pBI-mERS162F. In average, hypocotyl has a higher transformation than cotyledon. Evidences from PCR identification and Southern analysis have demonstrated the integration of the transgenes in transformants. Transformation rate estimated from antibiotic selection varied from 0 to 5.7% depending on the types of explant and T-DNA. However, through Southern hybridization, it is found that multi-copies integration and DNA rearrangements have been occurred in most transformants. Morphological and characteristic alternation such as slower in plant growth, shorter in plant height, easy branching and late bolting were noted. Presently, only one line with a slight delay in the yellowing of florets was obtained. Further studies on the transgene expression and the transgenic progenies are undergoing.

P-2014

Switchgrass Transformation by Microprojectile Bombardment with pAHC25 a GUS-BAR Construct. J.K. MCDANIEL, Z. Tomaszewski, V. Rudas, and B.V. Conger. Dept. of Plant and Soil Sciences. The University of Tennessee. Knoxville, TN 37901. Email: congerbv@utk.edu

Embryogenic calluses of switchgrass (*Panicum virgatum* L.) derived from immature inflorescences were bombarded with tungsten particles coated with the plasmid pAHC25. The plasmid contains the selectable *bar* (Basta(r)) gene and the reporter *uid*A (GUS) gene. Transient GUS expression was detected in callus tissue 48 h after bombardment. Plants were generated from cultures grown on MS medium with 10 mg l^{-1} bialophos. GUS expression was observed in the pollen, ovaries, and lodicules of transgenic plants. Eighty-nine Basta tolerant plants were obtained from the experiments. Presence of the *bar* and *uid*A genes was confirmed by Southern blot hybridization. Crosses between transgenic and nontransgenic plants resulted in Basta tolerant T_1 plants indicating inheritance of the *bar* gene. Supported by USDOE through UT-Battelle LLC and Oak Ridge National Laboratory under Contract No. 11X-SY 161C.

P-2015

Genetic Transformation of Switchgrass Mediated by *Agrobacterium tumefaciens*. M.N. SOMLEVA and B.V. Conger. Department of Plant and Soil Sciences, University of Tennessee, Knoxville, TN 37901-1071. Email: congerbv@utk.edu

The use of *Agrobacterium* for gene transfer in economically important forage grasses is limited. Switchgrass is a warm season perennial C_4 grass that has potential as a bioenergy crop as well as forage. Genetically transformed switchgrass plants were produced by cocultivating somatic embryos, embryogenic calluses, plantlet segments, and mature caryopses with a disarmed *A. tumefaciens* strain AGL 1, which harbors the binary vector pDM805 containing the beta-glucuronidase (*gus*) gene and a selectable marker, the phosphinothricin acetyl transferase (*bar*) gene. Various factors were found to influence the T-DNA delivery efficiency. These include preinduction of morphogenetic potential in target tissues and the presence of acetosyringone during inoculation and cocultivation. The inoculated explants were selected on medium with 10 mg L^{-1} bialaphos and the resultant plantlets were treated with the herbicide Basta. Approximately 600 transgenic plants were produced and the transformation efficiency was 14-24% for somatic embryos and calluses. Stable integration and expression of the transgenes in T_0 plants were confirmed by molecular analyses. Most of the tested transformants contained 1-2 copies of the insert. Research was supported by the University of Tennessee-Battelle LLC under Contract No.11X-SY161C.

P-2016

Transformation Process Exacerbates Cytological Variation in Transgenic Grass and Cereal Plants. H.W. CHOI, P.G. Lemaux, and M.-J. Cho. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: choihw7@uclink4.berkeley.edu

Plant transformation technology has been an important tool for improving major crop species through the introduction of desirable traits. We have recently reported high frequencies of cytogenetic abnormalities in transgenic barley and oat plants, compared with their nontransgenic plants: barley (46% vs. 0-4.3%) and oat (58% vs. 0-14%). In this study we further investigated the cytological status of transgenic plants of wheat, orchardgrass and tall fescue. Analysis of cytological studies indicated that even higher cytological variation occurred in transgenic plants of wheat, orchardgrass and tall fescue compared with their nontransgenic plants: wheat (36% vs. 0%), orchardgrass (70% vs. 0%) and tall fescue (50% vs. 17%). The most common cytological variation in transgenic hexaploid species (2n x 2), oat, wheat and tall fescue, was aneuploidy, followed by deletion of chromosome segments; no changes in ploidy level were observed. In contrast, ploidy changes were a major cytological variation in diploid transgenic barley (2n x 4) and tetraploid transgenic orchardgrass (2n x 8) plants. Our data indicate that additional stresses imposed by the transformation process over those encountered with *in vitro* culture alone affect cytological variation in transgenic plants. Another conclusion from these studies is that the nature of the chromosomal aberration, *e.g.*, strict ploidy changes or aneuploidy, appears to be dependent upon the particular plant species and its fundamental genomic state.

P-2017

Use of Cyanamide Hydratase Gene as a Selectable Marker for the Transformation of Sorghum. J. JAYARAJ, H.Yi, A. Anand, T. Weeks*, G.H. Liang, and S. Muthukrishnan. Department of Agronomy and Biochemistry, Kansas State University, Manhanttan, KS 66502.* USDA-ARS, University of Nebraska, Lincoln, NE. E-mail: jaya@ksu.edu

The selectable markers that are commonly used in plant transformation compromise of genes conferring resistance to herbicides or antibiotics. These genes have a potential to be transferred to non-target crops or weed species and thus cause undesirable effects in weed control. This necessities the evolution of new selectable markers that are safer and efficient for plant transformation. We have used a selectable marker, Cyanamide hydratase gene (*cah*) for transformation of sorghum with certain pathogenesis related protein (PR) genes. The PR-protein genes encoding wheat chitinases, glucanases and rice thaumatin-like protein were cloned into the pCAH plasmid harboring the *cah* gene. The resulting constructs were used for transformation of embryo-derived calli of sorghum. The transformed calli were grown in culture medium amended with cyanamide (12.5mg/l). The concentration of cyanamide was gradually increased to 25 and 50 mg/l during the course of selection. The regenerated putative transgenic plants were further tested for the presence of transgene by PCR analyses and 6 plants showed the presence of the *cah* gene. The *cah* gene appears to be a useful selectable marker for sorghum transformation.

P-2018

An Efficient System for Transformation and Plant Regeneration of Sorghum Using Highly Regenerative, Green Tissues. M.-J. CHO and P.G. Lemaux. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: mjcho@nature.berkeley.edu

Lack of effective *in vitro* culture systems for sorghum [*Sorghum bicolor* (L.) Moench] is one of the major barriers to the improvement of sorghum through genetic engineering. In this study we developed a very reliable and efficient system for maintaining highly regenerative, green cultures over extended periods. Cultures, derived from immature scutellar tissues of a sorghum cultivar (Texas 430), were grown on callus-induction media containing different combinations of 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP) and cupric sulfate under dim light conditions. The addition of copper in combination with a low level of BAP (0.044 μM) or without BAP resulted in the efficient induction of highly regenerative, green tissues. The resulting tissues produced multiple green shoots and could be maintained for more than a year without marked loss in regenerability. The use of these improved culture and regeneration protocols resulted in successful transformation of Texas 430 with 8.0% transformation frequency; from 87 independent explants, 7 independent events were obtained after a 9- to 16-week selection period with 30 mg/L of hygromycin B for hygromycin resistance. Presence of transgene(s) in T_0 sorghum plants was confirmed by PCR. DNA blot hybridization and analysis of functional transgene expression are in progress.

P-2019

High-Frequency Transformation of Rice (*Oryza sativa* L.) via Microprojectile Bombardment of Mature Seed-derived Highly Regenerative Tissues. M.-J. CHO, H. Yano, D. Okamoto, V.K. Le, K.L. Newcomb, B.B. Buchanan, and P.G. Lemaux. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: mjcho@nature.berkeley.edu

A highly efficient and reproducible transformation system for rice (*Oryza sativa* L. cv. Taipei 309) was developed using microprojectile bombardment of highly regenerative tissues. These tissues were induced from mature seeds on NB-based medium containing 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), and high cupric sulfate under dim light conditions; germinating shoots and roots were completely removed. Highly regenerative tissues were proliferated on the same medium and used as a transformation target. From 431 explants bombarded with transgenes, e.g., wheat thioredoxin *h* (*wtrxh*) and β-glucuronidase (*uidA*; *gus*) genes, and a hygromycin phosphotransferase (*hpt*) gene, 25 independent transgenic events were obtained after an 8- to 12-week selection period for hygromycin resistance, giving a 5.8% transformation frequency. Of the 25 independent events, 14 (56%) were regenerable. Coexpression of the other introduced transgene(s) was detected in 75% of the transgenic clones. Stable integration and expression of the foreign genes in T_0 and T_1 plants were confirmed by polymerase chain reaction (PCR) amplification and DNA hybridization and western blot analyses.

P-2020

Long-term Stability of Transgene Expression Driven by Barley Endosperm-specific Hordein Promoters in Transgenic Barley (*Hordeum vulgare* L.) Plants. H.W. CHOI, P.G. Lemaux, and M.-J. Cho. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. E-mail: choihw7@uclink4.berkeley.edu

In order to evaluate the stability of transgene expression driven by the B_1- and D-hordein promoter in transgenic barley (*Hordeum vulgare* L.) plants, we analyzed 15 independent transgenic barley lines (6 for *uidA* and 9 for *gfp*) produced via microprojectile bombardment of immature embryos; 4 lines were diploid and 11 were tetraploid. The expression and inheritance of transgenes were determined by analyses of functional transgene expression, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH). Ability to express transgenes driven by either B_1- or D-hordein promoter was stably inherited in T_4 and later generations: T_4 (7 lines), T_5 (5 lines), T_6 (1 line), T_7 (1 line) and T_8 (1 line). Homozygous transgenic plants were obtained from seven lines (2 for *uidA* and 5 for *gfp*); the remaining lines are currently being analyzed. The application of the FISH technique for physical mapping of chromosomes was useful for early screening for homozygous plants by examining for the presence of the transgene. For example, one line expressing *uidA* and shown to have fluorescence signal on both chromosomes was confirmed as a homozygous line by transgene segregation ratio; additionally this line showed stable inheritance of the transgene to T_8 progeny. The expression of transgenes in most lines (13 out 15 lines) was stably transmitted to T_4 or later generations, although transgene silencing was observed in some progeny in the remaining two lines.

P-2021

Defining Optimal Storage Conditions for Cotton Tissues Prior to Ovule Culture. B.A. Triplett* and D.S. JOHNSON**. *USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124; **Student Intern Program, Xavier University, New Orleans, LA 70125. Email: btriplet@nola.srrc.usda.gov

Cotton ovule culture has proven to be a useful model for studying cotton fiber development in many laboratories around the world. Typically, flowers are harvested on the day of anthesis, and the dissected ovules are immediately transferred to culture conditions. Since the number of available flowers per day can be limited from greenhouse-grown plants in the winter months, we have tested conditions for storing ovules prior to culture initiation. Flowers were harvested on the day of anthesis and floral parts were removed to reveal the carpel. Groups of carpels were placed in sealed specimen cups and stored at 4°C until culture initiation. Replicate ovule cultures were initiated from bolls after 1 to 14 days of storage at 4°C using our standardized culture procedures. Control cultures were initiated with freshly harvested tissues. Cultures were grown for 21 days at 32°C, photographed for visual comparison, and the fresh weight of tissue produced during this period was determined. Ovules with adhering fiber were dried *in vacuo* and the fiber was separated from the ovules. The cellulose content of fiber from each set of cultures was determined using the Updegraff method. Ovules could be stored for up to 10 days at 4°C without deleterious effects on fresh or dry weight accumulation. While the total amount of fiber produced by the cultures decreased with storage periods of over 10 days, the cellulose content of the fiber that was produced was comparable to control cultures. These storage conditions have proven useful for our experiments in which a large number of replicate cultures must be established simultaneously, for shipment of ovules to collaborators' laboratories at distant locations, and will be useful for pre-launch conditions for microgravity experiments that are planned for the Space Shuttle.

P-2022

Cryopreservation of embryogenic avocado (*Persea americana* Mill.) cultures. D. EFENDI[1], R.E. Litz[1], and F. Al Oraini[2]. [1]Tropical Research & Education Center, University of Florida, 18905 SW 280 St., Homestead, FL 33031-3314; [2]National Agriculture and Water Research Center, P.O. Box 10939, Riyadh 11443, SAUDI ARABIA. Email: rel@mail.ifas.ufl.edu

The continuous availability of embryogenic avocado cultures is essential for genetic transformation and other in vitro studies. Maintenance of embryogenic avocado cultures is strongly genotype- and cultivar-dependent, and loss of embryogenic competence can occur as early as 4 months following induction. Cryopreservation can assure the long-term storage of embryogenic cultures, and can obviate the need for the tedious annual renewal of embryogenic cultures at the time that trees are flowering. Embryogenic avocado cultures have been successfully stored in liquid nitrogen with a cryoprotectant consisting of 5% glycerol and 5% DMSO using the Nalgene Cryo 1oC Freezing Container cryoprotection system.

P-2023

Shipping Procedures for Plant Tissue Cultures. BARBARA M. REED, C.L. Paynter, and B. Bartlett. USDA-ARS National Clonal Germplasm Repository, Corvallis, OR 97333-2521. Email: reedbm@bcc.orst.edu

Germplasm repositories collect, maintain, evaluate, and distribute plant materials to interested scientists throughout the world. International shipping of in vitro cultures is often more successful than distribution of other plant forms because the cultures are more likely to comply with quarantine regulations. Seasonal availability of scion wood or rooted cuttings may limit their usefulness for germplasm distribution. Transportation of sterile cultures can be challenging as well. Maintaining sterility within the container, liquefaction of the medium due to shaking or cabin pressure changes during flights, freezing or overheating, and neglect on shipping docks for extended periods are a few of the difficulties. The National Clonal Germplasm Repository in Corvallis distributes 200-500 plant tissue cultures to national and international requestors each year and has developed procedures for distribution that minimize these common problems. Shoot cultures are transported in sealed, semi-permeable plastic bags that are carefully folded and packed in crushproof containers. Sealed tissue-culture bags eliminate the contamination threats posed by air pressure changes or movement of the growth medium into the caps that can be a problem with tubes or jars. Firm medium (7-8 $g \cdot L^{-1}$ agar), careful folding, and packing of the bags in crushproof boxes with adequate packing materials minimize the shifting of plants and medium in transit. Special attention to weather conditions en route and timely alerting of the recipient prior to the arrival date decreases the number of shipments lost due to freezing, overheating, or long delays in customs or quarantine offices. Cultures remain viable for a month or more at room temperature when properly packed and shipped, and usually result in healthy cultures even after lengthy transit or customs delays.

P-2024

Overcoming of Interspecies Incompatibility in the Solanaceaous Genera Nicotiana and Capsicum via In Vitro Techniques. V.M. NIKOVA[1], R.D. Vladova[1], A.C. Petkova[1], and A. Iancheva[2]. [1]Institute of Genetics, BAS, Sofia 1113, [2]Complex Experimental Station, 6300 Haskovo, BULGARIA. Email: vnikova@bas.bg and r.pandeva@netcourrier.com

Interspecies hybridization is often accompanied with barriers of incompatibility, which are activated in different stages. Within the genera *Capsicum* and *Nicotiana*, they acted immediately after pollination (*C. eximium* x *C. annuum*), in proembryonal *stage (C. praetermissum x C. annuum), in the process of embryo differentiation (C. annuum x C.* praetermissum, *C. annuum* x *C. eximium*, *C. baccatum* x *C. annuum*) as well as in the development of F_1 hybrids and the formation of their generative organs (*N. plumbaginifolia* x *N. tabacum* and *N. sylvestris* x *N. tabacum*). Different in vitro techniques were applied in order to produce viable F_1 hybrid plants or to improve their fertility, e.g., embryoculture method for three *Capsicum* interspecies combinations and tissue culture method for two *Nicotiana* F_1 hybrids. Embryos in globular up to cotyledon stage of the crosses *C. annuum x C.* praetermissum, *C. annuum* x *C. eximium*, *C. baccatum* x *C.annuum* were successfully grown on MS agar medium, supplemented with 0,0.5 mg/L giberrellic acid, kinetin and NAA, respectively. Stem cuttings of the plantlets obtained were additionally micropropagated on MS medium for rooting with 2 mg/L ferulic acid. Stem pit parenchyma of *N. plumbaginifolia* x *N. tabacum* and *N. sylvestris* x *N. tabacum* F_1 hybrids was cultured in vitro on MS solid medium. Two parallel series of supplements were tested: 1) TDZ (0.5, 1.0 mg/L) + IAA (0.5, 1.0 mg/L) + GA_3 (0.5, 1.0 mg/L) for callus and organ initiation and 2) KIN (0.5 mg/L) + NAA (2 mg/L) for callusogenesis and KIN (2 mg/L) + NAA (0.5 mg/L) for organogenesis. TDZ-combination stimulated moderate callus and organ formation and did not improve the fertility of the regenerants (R_1) obtained. KIN-containing media affected intense callus growth and abundant organogenesis. R_1 obtained after longer cultivation (5th passage for *N. plumbaginifolia* x *N. tabacum* and 6th passage for *N. sylvestris* x *N. tabacum*) were male sterile but with restored female fertility and formed seed capsules after backcrossing. An electophoretic analysis of seed proteins from *N. sylvestris, N. plumbaginifolia, N. tabacum*, F_1 and R_1 were performed.

P-2025

In vitro Culture of Sea Thrift (*Armeria maritima*). P.T. LYNCH[1], L. Brewin[1], A. Mehra[1], and M.E. Farago[2]. [1]Centre for Environmental and Applied Science Research, University of Derby, Kedleston Rd., Derby, DE22 1GB, UK. [2]EGRG, T H Huxley School of the Environment, Imperial College, Prince Consort Rd., London SW7 2BP, UK. E-mail: P.T.Lynch@derby.ac.uk

Armeria maritima, generally grows in the UK on non-metal contaminated coastal sites. However, its ability to grow in a copper-enriched bog (Dolfrwynog Bog) in Wales, which was a site of metal extraction in the last century suggests that *A. maritima* could be an indicator species of contaminated land. Previous studies on bioavailability and uptake of copper by *A. maritima* in Dolfrwynog Bog showed that high levels of copper are taken up and accumulated by this plant, indicating that it is able to thrive in these conditions because of internal tolerance mechanisms. As part of ongoing studies into these tolerance mechanisms and towards the development of copper hyperaccumulating *A. maritima* lines, *in vitro* culture protocols have been developed. The influence of naphtaleneacetic acid (NAA), 6-benzylaminopurine (BAP) in Murashige and Skoog (MS) medium containing 30g/l sucrose, 0.5mg/l Plant Preservative Mixture (PPM), and 8g/l agar on plant regeneration from leaf explants has been assessed. To maximise shoot regeneration the optimum concentrations were 0.1 mg/l NAA and 0.1 mg/l BAP. The inclusion of PPM in the medium was essential to avoid microbial contamination. The concentration of MS salts in media without growth factors significantly influenced the rooting of *A. maritima*. *In vitro* derived plants are being established in compost. Histological studies are being undertaken to determine the site of origin of regenerated shoots.

P-2026

A New Approach for In Vitro Regeneration of Phaseolus Vulgaris. M. MUMINOVA, M. Nasretdinova, and S. Djataev. Department of Plant Biotechnology, Institute of G&PEB of Tashkent, UZBEKISTAN. Email: magfrat@usa.net

Any such transformation system must satisfy a number of conditions, including, gene transfer to a sufficient number of cells in the explant which will be or become involved in the morphogenic events leading to the regenerated plant. Genotype, explant choice and the culture protocol have a decisive influence on the quality of the obtained regenerating structures. We studied the possibility to obtain somatic embryogenesis on the cotyledons of immature seeds of Phaseolus vulgaris. It was established that the process of regeneration depend on glutamine. It was shown that addition 250 mg/l glutamine to the basal medium M&S induced embryogenesis.

P-2027

Development of Shoot Culture Protocols for Eastern Black Walnut (*Juglans nigra*). M.J. BOSELA and C.H. Michler. USDA Forest Service, North Central Research Station, Hardwood Tree Improvement and Regeneration Center, 1159 Forestry Building, Purdue University, West Lafayette, IN 47907-1159. Email: mbosela@fnr.purdue.edu

Non-lignified coppice shoots collected from seedling rootstocks after grafting were used as a source of explants for culture initiation. Following bleach sterilization, the explants (shoot tips and nodal cuttings) were transferred individually to culture tubes of hormone-free media for 14-18 days. Explants that appeared visually sterile were subsequently transferred to vessels of experimental media, differing in salt formulation (MS, DKW, and WPM) and cytokinin type (0, ZEA, BA, and TDZ). The cultures were maintained under a 16 hr photoperiod and subculture every 3-4 wks. Across all shoot lines and cytokinin types, the cultures appeared least stressed and most normal in phenotype on DKW media. On MS media the shoots elongated well, but were frequently chlorotic. Shoots cultured on WPM were often dark green, but they exhibited symptoms of tissues stress, such as hyperhydricity, anthocyanin synthesis, partial leaf necrosis, callus development from the shoot base. Shoot growth was less on WPM than on DKW or MS and generally ceased after 2-3 culture periods. Although most of the initial explants elongated on hormone-free media, continuous growth was not possible without exogenous cytokinins. BA and ZEA (at 5 uM) both supported shoot elongation but spontaneous axillary branching was not observed. On TDZ media (0.05 to 0.1 uM) axillary branching was induced, but the resultant shoots were aberrant in phenotype and they frequently failed to elongate following subculture. Hyperhydricity, stem hypertrophy (swelling), leaf epinasty, and leaflet underdevelopment were associated with the use of TDZ. Fasciated shoots were regenerated at low frequencies on TDZ media (< 5%), but not on media with ZEA or BA. The severity of the phenotypes seen on TDZ media was related to the distance between the axillary meristem and the culture media. Shoots developing at or below media level showed the most severe phenotypes.

P-2028

Plant Regeneration from Sugarcane Seed-derived Callus. K. CHENGALRAYAN*, A. Abouzid, and M. Gallo-Meagher. Agronomy Department, University of Florida, Gainesville, FL 32611-0300. Email: chengal@ufl.edu

Sugarcane accounts for approximately 70% of the world's sugar and is an economically important cash crop in the tropical and subtropical regions of many countries. Due to its global importance, much research has focused on sugarcane crop improvement through plant breeding, and more recently through biotechnology. Effective utilization of biotechnological approaches such as the isolation of somaclonal variants, protoplast fusion and genetic transformation, rely on efficient and reliable regeneration systems. Sugarcane tissue culture was first initiated in Hawaii in 1961, and subsequently several protocols for somatic embryogenesis and organogenesis have been developed using callus derived from various explants like immature inflorescences, young leaves, and apical meristems. There are limitations related to use of these explants. These include the need to maintain excessive greenhouse plantings and seasonal dependence. In this report, we describe a protocol for plant regeneration from seed-derived callus. Sugarcane (Saccharum spp. hybrid cv. CP84-1198) seeds were cultured on modified Murashige and Skoog (MS) basal medium supplemented with 1, 3, 5 and 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), picloram or thidiazuron (TDZ). Percent germination, percent callus induction, amount of callus produced per seed, and the time required to regenerate embryogenic callus were determined. Plants were regenerated from seed-derived callus by either transferring to modified MS medium devoid of growth regulators or with 2.5 mM TDZ.

P-2029

Factors Affecting Micropropagation of *Asimina tetramera*, an Endangered Florida Scrub Species. J.R. CLARK and V.C. Pence. Center for Research of Endangered Wildlife (CREW), Cincinnati Zoo and Botanical Garden, Cincinnati, OH 45220. E-mail: johnclark74@yahoo.com

Asimina tetramera (Annonaceae), an endangered Florida scrub species, is being grown at Bok Tower Gardens (Lake Wales, FL) as part of the Center for Plant Conservation's National Collection of Endangered Plants. Efforts to propagate this species in vitro are being made as part of a program at CREW aimed at applying tissue culture techniques to difficult-to-propagate endangered US species. Nodal explants from greenhouse-grown seedlings were used to establish culture lines in vitro. Shoot cultures were maintained using semi-solid MS medium with 0.1 mg/l BAP and 0.01 mg/l NAA. Shoot multiplication rates were low, however, and phenolic oxidation markedly affected shoot proliferation. Incorporation of citric acid (1 mg/l) and polyvinylpolypyrrolidone (PVPP; 500 mg/l) reduced visible oxidative effects and culture vigor improved. Other factors affecting culture improvement included increased light intensity and frequency of subculturing. Root initiation has not yet been successful using standard procedures and the affects of auxin concentration, antioxidants and other culture conditions are currently being examined. This research is funded, in part, by the Institute of Museum and Library Services grant no. IC-00034-00.

P-2030

An Alternative Propagation Method of Bergenia Ligulata Through Leaf Culture. S. MALLA and P. Malla. Armit Science Campus, Tribhuvan University, Kathmandu, NEPAL, Department of Microbiology, Trichandra Campus, Tu Kathmandu, NEPAL. Email: Prakash_malla@hotmail.com

The rhizome of medicinal plant Bergenia *ligulata* is widely used in Nepal and in India. Harvesting of the plant rhizomes for sale has been destructive and widespread, continued it will hasten extinction. A micropropagation scheme for *Bergenia* was developed using leaf cuttings as explants. In the first step, adventitious shoots were regenerated from leaf explants. For this purpose, it was necessary to apply a treatment with antioxidants or absorbing agents to prevent tissue browning. These shoots were subsequently used to set up a micropropagation system and production of uniform plants. Multiplication through shoot culture was not effective, but the use of nodal explants was most suitable. A total of 180 factorial combination of plant regulators was screened for shoot efficiency. The use of BAP with a small amount of IAA resulted in high multiplication factors that allow the production of a large amount of shoots. However, presence of IAA in the medium altered the frequency of shoot multiplication. Best rooting was readily achieved upon transferring shoots into MS medium with IAA or IBA. They could be easily acclimatized to greenhouse condition and further transferred to the field. None of the plants showed any phenotypic variation. The method thus offers the possibility to multply selected elite clones of *Bergenia ligulata* and to provide plantlets for controlled field cultures. This might help to avoid the excessive collection of plant material from natural habitats.

P-2031

Somatic Embryogenesis, Secondary Somatic Embryogenesis, and Shoot Organogenesis in Rosa hybrida and Rosa chinensis minima. XIANGQIAN LI, Sergei F. Karsnyanski, and Schuyler S. Korban. Department of Natural Resources & Environmental Sciences, University of Illinois, Urbana, IL 61801. E-mail: xli7@uiuc.edu

The influence of various 2,4-D concentrations (11.3 to 181 μ M) on callus induction from leaf tissues of *Rosa hybrida* cvs. Carefree Beauty and Grand Gala and *R. chinensis minima* cv. Red Sunblaze was evaluated. Following transfer of callus to a regeneration medium containing different concentrations of TDZ (0 - 90.8μ M), BA (0 - 44.4μ M), or GA3 (2.9μ M), alone or in various combinations, the highest frequency of embryogenic (32%) and organogenic (56%) callus was induced on 'Carefree Beauty'. Secondary somatic embryos were also induced on somatic embryos of 'Carefree Beauty'. The effects of different concentrations of TDZ (2.3μ M), BA (2.2-4.4 μ M), and ABA (3.8-7.6μ M), alone or in combination, on proliferation and germination of secondary somatic embryos were also evaluated. ABA was found to be the most effective in promoting proliferation and germination of somatic embryos. The size of secondary embryogenic callus grown on ABA increased by 36-fold, while germination of these embryos was more than five times compared to those grown on BA or TDZ. For *R. chinensis minima* cv. Red Sunblaze, only somatic embryogenesis (6.6%) was observed; while, for *R. hybrida* cv. Grand Gala, only shoot organogenesis (3.3%) was observed.

P-2032

Maturation and Germination of Somatic Embryos from Three Distinct Cultivars of Rose. J. Castillon, B. Jones, and K. KAMO. Floral & Nursery Plants Research Unit, National Arboretum, USDA, Beltsville, MD 20705. Email: kkamo@asrr.arsusda.gov

Many cultivars of rose produce large numbers of somatic embryos, but the germination rates of these embryos vary widely between cultivars and in most cases have been too low for development of efficient transformation protocols. Embryogenic callus of three cultivars of rose, the Floribunda cv Trumpeter, the Multiflora rootstock cv Dr. Huey and the Hybrid Tea cv Tineke, were used to study somatic embryo maturation and germination. Globular stage embryos were first isolated from the callus by washing and filtering the callus cells. This procedure provided embryos at the same develomental stage for the maturation and germination studies, and the washing/filtration procedure itself significantly improved germination rates for both Trumpeter and Tineke. Maturation and germination rates were evaluated in response to sucrose, glucose, fructose, or maltose as the primary carbon source and in response to various concentrations of either myo-inositol, polyethylene glycol, or mannitol in combination with sucrose. The optimum germination rate achieved was 27% for Trumpeter, 36% for Dr. Huey, and 13% for Tineke.

P-2033

Histology and Scanning Electron Microscopy of Somatic Embryo Development in Grapevine. S. JAYASANKAR, B.R. Bondada, Z. Li, and D.J. Gray MREC, IFAS, University of Florida, 2725, Binion Road, Apopka, FL 32703.

Somatic embryo development in grapevine was studied using histology and scanning electron microscopy. These studies confirmed the presence of a suspensor, indicating the probable single cell origin of these somatic embryos. The suspensor consisted of several files of cells, varied significantly in length among the somatic embryos of various cultivars and was persistent even in fully developed somatic embryos. In contrast, the suspensor in a mature zygotic embryo was rudimentary and comprised of only two or three files of cells. In addition, our studies show that the formation of meristematic dome in grapevine somatic embryo occurs as early as heart stage of development. Histological observations revealed that the meristematic dome is 4-6 cell layers deep and are rich in protein. In some cases, there was high vacuolation of cells in the apical meristem and in these somatic embryos the dome comprised of only 2-3 cell layers.

P-2034

Influence of UV Rays on Pepper (Capsicum annum L.) Cultivated In Vitro. N. ZAGORSKA1, V. Sotirova1, S. Daskalov1, B. Dimitrov1, V. Lapshin2, R. Butenko2, and I. Kozareva3. 1. D. Kostoff Institute of Genetics, BASS, Sofia 1113, BULGARIA. 2. K. a. Timiriazev Institute of Plant Physiology, RAS, Botanitcheskajia 35, 127-173 Moskow, RUSSIA. 3. Horticultural Sciences Department, University of Florida, Gainesville, FL 32611. E-mail: zagorska@bas.bg

The objective of this work was to study the influence of UV rays on callus, shoots and isolated meristems of pepper (varieties: Zlaten medal, Fitostop, Curtovska kapija, Orangava kapija, Albena and Pirin and lines: Borijana, Slavijanka, Svetla and Vibo) cultivated *in vitro* and irradiated with high doses of UV rays. The results showed that plant height, leaf and fruit shape, isolated meristem variability, etc., depend on UV irradiation dose and genotype. Higher dose (1.66 W/m^2) inhibits strongly morphogenesis. The Bulgarian variety Pirin was the most resistant to UV rays followed by Vibo, Slavijanka, and Svetla. The inhibitory effect of UV rays was most severe at the early stages of shoot development and the early meristem cultivation. The regenerants were grown up to maturity. They differed morphologicaly from the control. Particularly great variations were observed in pepper variety Pirin. The fruits were significantly larger than the control and were situated upright in some of the plants. That trait was inherited in the next generation. The investigation of R1 of the regenerants of variety Pirin to the virus (TMV) and to *Phytophtora capsici* showed that 90% of the plants were resistant to TMV and 60% - to *Phytophtora capsici.*

P-2035

Gene Introduction Method Affects Transgene Expression in Chrysanthemum (*Dendranthema grandiflora*). J.A. TEIXEIRA DA SILVA and S. Fukai. Department of Floriculture, Kagawa University, Miki-cho, Kagawa, 761-0795, JAPAN. E-mail: jaimetex@angelfire.com

Studies aimed at improving the transformation efficiencies of standard and spray-type chrysanthemum (*Dendranthema grandiflora* (Ramat.) Kitamura) were conducted, with tobacco serving as an unrelated control. Trials to optimize the *in vitro* regeneration systems and to maximize the transient transgene (*uidA*) expression were performed on *in vitro* and greenhouse-derived stem segments as explants. Four different gene introduction methods - particle bombardment, *Agro*infection, sonication-assisted *Agrobacterium* transformation (SAAT) and Agrolistics (bombardment + Agroinfection) - were tested. In the latter three, pBI121 or p-SKGN1 (intron- containing novel plasmid construct) - both containing the *uidA* and *nptII* genes under the control of a CaMV-35S promoter and harboured in *A. tumefaciens* LBA4404 - were utilized, while in particle bombardment pKT_2 was utilized. Putative transformants (PTs) - harvested off a 25mg/l kanamycin selective medium - obtained from any of these gene introduction methods showed highly localized leaf tissue expression, although expression could be detected in almost all leaf tissue types (independent of age and cultivar): leaf tip, veins and midrib, leaf edge, intervein and epidermis. The most predominant stable GUS expression was in the midribs and veins of physiologically older, basal leaves. A preliminary analysis of the stable GUS expression and molecular (PCR)-derived transformation efficiencies (TrEs) of PTs indicate that: a) TrEs are affected by the gene introduction method; b) TrE is cultivar-dependant. The choice and optimization of the regeneration and gene transfer protocols are vital for predictability of transformation success and efficiency in this important floricultural crop.

P-2036

Comparative Effect of BAP and TDZ on Multiplication of Micropropagated Saffron (Crocus sativus L.) Corms. S. Blázquez, A. Piqueras, C. Rubio and J.A. Fernández. Area de Genética, Dept. Ciencia y Tecnología Agroforestal, E.T.S.I.A. Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071 Albacete, SPAIN, Email: jafernandez@idr-ab.uclm.es (S.B., C.R., J.A.F.), and Dept. Nutrición y Fisiología Vegetal, C.E.B.A.S. (C.S.I.C.), 3080 Murcia, SPAIN. E-mail: piqueras@natura.cebas.csic.es (A.P.)

Experiments were performed to improve the regenerative efficiency beyond stage II of the protocol developed for saffron micropropagation and currently used at the E.T.S.I.A. (Albacete, Spain) laboratory. During the last three yr the combination of plant growth regulators, used for the propagation and multiplication of nodular cormogenic calli of saffron, was based on the exclusive application of BAP and 2,4-D during stage II. When these regulators were used, only a 20% of the nodular cormogenic calli developed morphogenic structures able to produce leaves and become independent corms. Since the objective of our work is to develop a mass propagation system for saffron, higher rates of morphogenesis would be desirable in our stage II cultures. To achieve this, several concentrations of the cytokinin-like regulator TDZ and BAP were tested in nodular cormogenic stage II cultures, to evaluate its capacity to induce the formation of independent microcorms with developed leaves or leaf primordia. Our results show that TDZ (0.1 mg/l) with a 60% of regenerants is significantly more efficient for the production of microcorms with fully developed leaf primordia than BAP (2 mg/l) with only 20%. By incorporating TDZ we expect to accelerate the recovery of completely developed plantlets for rooting and ex vitro acclimatization. These results will be used to improve the current micropropagation protocol for saffron as a preliminary step for the scale-up towards a mass propagation system using bioreactors.

P-2037

Micropropagation of Triploid Crossandra. M. GANGA, N. Chezhiyan, and K.A. Shanmugasundaram, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, INDIA. E-mail: gangahort@usa.net

The crossandra (Crossandra infundibuliformis Salisb.) is regarded as a high value flower crop, the flowers being valued for their bright colours, lightweight and good keeping quality. The triploid crossandra does not set seeds and is conventionally propagated by cuttings which is a very cumbersome and time consuming process with very low rates of multiplication, which are not efficient enough to meet the ever increasing demands for quality planting materials. Considering the existence of an imperative need to standardize a reliable protocol for in vitro propagation of the triploid crossandra, direct and indirect organogenesis were attempted through shoot tip culture and leaf bit culture, respectively. The culture media involved were full strength MS (Murashige and Skoog, 1962) medium, 1/2 strength MS medium or SH (Schenk and Hilderbrandt, 1972) medium fortified with various growth regulators. Induction of multiple shoot proliferation from shoot tips involved the cytokinin BAP (Benzyl Amino Purine) alone or in combination with a gibberellin (GA_3) or an auxin (NAA -Naphthalene Acetic Acid), whereas, induction of callus masses from leaf bit explants involved the auxin NAA alone or in combination with either of the two cytokinins, BAP or KIN (Kinetin). The medium for regeneration of shoots from leaf bit derived callus involved inclusion of the amino acid L-proline. The best response to multiple shoot regeneration from shoot tips was recorded on full strength MS medium fortified with BAP alone at 1.0 mgl^{-1}. The maximum response to callus induction from leaf bits was recorded on full MS medium fortified with 3.0 mgl^{-1} 2,4-D + 0.5 mgl^{-1} BAP and the maximum shoot regeneration from callus was recorded on 1/2 MS medium supplemented with 2.0 mgl^{-1} BAP + 600 mgl^{-1} L-proline.

P-2038

Effect of the Substituted Chromosomes Upon Developmental Processes In Vitro in 20 Wheat Lines. V. Chardakov, A. Dryanova, N. Tyankova, N. Zagorska, and B. Dimitroff. Institute of Genetics, Bulgarian Academy of Sciences, Sofia 1113, BULGARIA. E-mail: chardakov_vasil@yahoo.com

20 different wheat lines of cultivar *Chinese Spring*(CS) with substituted chromosome pair from cultivar *Timstein*(Tm) and the initial cultivars CS and Tm were used as an experimental material. The aim of the study was to indentify the chromosomes, which substitution provoke the most considerable effect upon dedifferentiative and developmental processes in vitro. Callus culture were induced from immature embryos. It was found that the intercultivar substitution doesn't reflect upon the callus induction and primary dedifferentiation. Description of the regeneration ability of the callus cultures was done according to two main indices: relative part of the calli, regenerating plants and coefficint of multiplication, as well as two complementary indices-number of the calli, producing rooths only and number of the fresh, nonembryogenic calli. The chromosomes with the most essential role were determined on the base of statistic assessment. The processes of regeneration are controled by genes, localized in 2A, 7A, 1B, 3B, 5B, 6B and 1D chromosomes. With the most positive effect upon the regenerative processes are 5B(Tm) and 2A(Tm) chromosomes. 5B and 1D chromosomes participate in the control on the regenerated plants number, as well as in the frequency of appearance of the embryogenic type calli.

P-2039

Changes in Polyamine Metabolism During the Acclimatization of Micropropagated *Populus* Plants. [1]J.L.CASAS-MARTINEZ, [1]M. Cortina, [1]M.D. Serna, and [1,2]A. Piqueras. [1]Unidad de Biotecnología Vegetal. Centro Iberoamericano de la Biodiversidad (CIBIO). Universidad de Alicante. P.O.Box 99. E-03080-Alicante SPAIN; [2]Departamento de Nutrición y Fisiología Vegetal. CEBAS-CSIC (Murcia-SPAIN). Email jl.casas@rn.ua.es

The objective of this research was to monitor the process of acclimatization of micropropagated plants at polyamine level using *Populus* as model system. To achieve this we followed the changes in total, free, conjugated (hydrolysed, acid-soluble) and bound (hydrolyzed, acid-insoluble) polyamine in *Populus* leaves during the different stages of the acclimatization to ex vitro conditions. These compounds showed a clear trend to decrease as acclimatization progressed. In fully acclimatizated plants, total polyamine content was 50% of *in vitro* plantlets. Free polyamine was the most abundant fraction present in leaf cells during acclimatization while the conjugated fraction was minority. The strongest variations were registered in the levels of bound polyamine fraction. From our results a clear relation between the rise in polyamines (spermidine and spermine) and the more stressful conditions during the initial stages of the acclimatization process was derived. Therefore, free polyamine titer could be used to assess the quality of current acclimatization protocols by providing a precise information useful to minimize the side effect imposed to micropropagated plantlets by the *ex vitro* environmental conditions.

P-2040

Development of Plant Regeneration and Genetic Transformation in the Papveraceae for the Metabolic Engineering of Benzylisoquinoline Alkaloids. S.-U. PARK, and P. J. Facchini. Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, CANADA. Email: spark@ucalgary.ca

We have developed the useful protocols of plant regeneration, genetic transformation and hairy root culture system in the Papaveraceae, which include the opium poppy (Papaver somniferum L.) and California poppy (Eschscholzia Cham.) for metabolic engineering in benzylisoquinoline alkaloid biosynthesis. Procedures have recently been developed in our laboratory for 1) Rapid protocol for high-efficiency somatic embryogenesis and plant regeneration from seed-derived embryogenic callus cultures of California poppy, 2) Improved somatic embryogenesis using embryogenic suspension cultures of California poppy, 3) An efficient Agrobacterium-mediated protocol for the stable genetic transformation of California poppy via somatic embryogenesis, 4) an efficient Agrobacterium-mediated protocol for the stable genetic transformation of intact opium poppy plants via shoot organogenesis, 5) The protocol for the establishment of transgenic opium poppy and California poppy root cultures using Agrobacterium rhizogenes, and 6) Metabolic engineering of benzylisoquinoline alkaloids in transgenic California poppy cell cultures. Modifications of cell secondary metabolism by genetic engineering may be important in producing higher levels of benzylisoquinoline alkaloids in California poppy cells. We present preliminary results from initial attempts to metabolically engineer benzylisoquinoline alkaloid biosynthesis in transgenic cell cultures. California poppy cell cultures show that cell lines transformed with constitutively expressed sense-BBE (berbine bridge enzyme), from opium poppy display an intense red-brown color compared to control cultures transformed with a 35S::GUS construct. In contrast, cell lines transformed with constitutively expressed antisense BBE from California poppy show virtually a complete loss of red-brown color. The benzophenanthridine alkaloids that accumulate in California poppy are typically orange to red in color; thus, our observations suggest that cell lines transformed with the sense - BBE construct accumulate more of these alkaloids, wheras cell lines transformed with anitsense-BBE accumulate little, if any, of the normal profile of benzophenanthirdine alkaloids. Our continuing research is focused on the development and characterization of this, and other, genetically-mediated metabolic manipulations of benzylisoquinoline alkaloid pathways in a variety of plant species.

P-2041

Development of Intergeneric Hybrids in Crop Brassicas via Embryo Rescue and Somatic Hybridization. G. RAVI KUMAR, S. R. Bhat, Shyam Prakash, and V. L. Chopra. National Research Center on Plant Biotechnology, Indian Agricultural Research Institute, Pusa Campus, New Delhi - 110012, INDIA. E-mail: grkmbio@yahoo.com

Many wild allies of crop brassicas in the Brassica coenospecies, group are potential donors of desirable nuclear and organelle encoded characters. To enlarge the genetic base for productivity traits and also for specific attributes like disease and pest resistance, tolerance to abiotic stress, specialty components of quality attributes and male sterility, the nuclear and organelle genes in the wild relatives of the cultivated species are of critical value. Efforts were made to develop novel genetic stocks in crop brassicas using sexual and somatic hybridization. Intergeneric hybrid between Erucastrum caraminoides (Webb ex Christ) O. E. Schulz (n=9), a wild species and Brassica nigra (Dwarf) (n=8) was obtained through ovary culture. Two somatic hybrids namely, Diplotaxis gomez-campoi (n=9) + Brassica nigra (Dwarf) (n=8), Sinapis pubescens (n=9) + Brassica nigra (n=8) were obtained following protoplast fusion. Hybridity of all of the hybrids was confirmed through RAPD and Isozyme markers. Molecular analysis was carried out for the cytoplasmic organelles to ascertain the mitochondrial and chloroplast status and chromosome analysis to study the meiotic behavior of these hybrids. These promising hybrids can act as a bridge species for transferring new genes from wild to crop species.

VT-2000

Establishment of a Human Hepatoma Cell Line, HLE/2E1, Suitable for Detection of P450 2E1-Related Cytotoxicity. I. NOZAKI and M. Namba. Department of Cell Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, Okayama 700-8558, JAPAN. Email: noz_7@yahoo.com.jp

By transfection of an expression vector of human cytochrome P450 2E1 (CYP2E1) into a human hepatoma cell line (HLE), a new cell line (HLE/2E1) that stably expresses activity of CYP2E1 had been established. The HLE/2E1 cell line expressed a higher level of CYP2E1 mRNA than did the mother HLE cell line. CYP2E1 enzyme activity determined by a p-nitrophenol oxidation assay was also higher in HLE/2E1 cells than in HLE cells. In addition, the enzyme activity of the HLE/2E1 cells was increased by ethanol treatment. Exposure to acetaminophen (APAP) or buthionine sulfoximine (BSO) caused a greater decrease in viability of the HLE/2E1 cells than that of the HLE cells, as determined by the MTT assay. The cytotoxicity of APAP or BSO to HLE/2E1 cells was inhibited by the addition of ethanol or vitamin E. However, the cytotoxicity of both APAP and BSO was enhanced by 24-h preincubation of HLE/2E1 cells with ethanol. These results show that this cell line provides a useful model for studying catalytic properties of CYP2E1 and cytotoxic mechanisms of chemical metabolized by CYP2E1.

VT-2001

Study of Embryonic Ploidy: a Probable Embryo Model. M.S. KUNDT and R.L. Cabrini. Department of Radiobiology, National Atomic Energy Commission, Buenos Aires, MD 1650 ARGENTINA. E-mail: KUNDT@CNEA.GOV.AR

The 2nd polar body (PB) studies in preimplantation mouse embryos were carried out to evaluate the possibility as reference cell to analyze ploidy. For that purpose embryos in a one cell stage [obtained by crossing hybrid females (CBAxC57BL) to NIH males] were cultured in vitro during 72 hs, individually fixed at morula stage and stained with Feulgen. The DNA content of 263 individual nucleus was evaluated cytophotometrically corresponding to 22 compact morulas of normal development. As haploid PB is present in all preimplatned stage, only embryos with one haploid nuclei were considered as normal. In 95.5% ($n = 21$) of the embryos the PB was present. DNA measurement of 21 PB was $1n \pm 0.1$. By the height sensibility of PB ploidy, the abnormalities were detected by the criterion of > 4.1 n and $< 1.9n$. The results showed that one embryo was completely haploid (1n). The rest of the embryos ($n = 20$) 222 blastomeres and 20 PB were analyzed. The DNA measurement showed that 92,7% of the blastomeres ($n = 206$) are between 2n and 4 n and 7.3 % showed ploidy anomalies, regarding the value n of their PB. The period of the cellular cycle was studied in the normal cell ploidy. This study showed that 16.5% of the blastomeres ($n = 34$) were in the period G1, 70.39% ($n = 34$) in the period S and 13.2% in the period G2 ($n = 27$). It is concluded that the PB study showed that it has properties as an excellent indicator of internal ploidía: it is present from the moment of the conception, easily recognizable in the perivitelin space in the embryo of one-two cells, remains in interface during the preimplantation development, it is haploid and digitalized pixel by pixel PB study showed the homogeneity of this type of cell, giving a reliable value of ploidy. The properties of the PB and the results showed that the PB could be an excellent indicator for embryonic ploidy studies on genotoxicity, maintaining its original ploidía during the preimplantation development while the blastomeres are susceptible to changes in its content of DNA starting from the first embryonic cleavage.

VT-2002

Three-dimensional Transgenic Model for Genotoxic Assessment Using Macroporous Cultispheres. D.N. FRAGA1, J.A. Jordan2, and S.R. Gonda3. 1University of Notre Dame, South Bend, IN 46637; 2Universities Space Research Association, Houston, TX 77058; 3Johnson Space Center, Houston, TX 77058. E-mail: Fraga.3@nd.edu

Human exposure to space environment imposes genetic hazards that must be identified and alleviated. Currently, a model test system is needed that is representative of cellular interactions in tissue, and capable of quantifying genetic damage induced by low levels of space radiation and chemicals. We describe a three-dimensional, multi-cellular tissue-equivalent model, produced by culturing genetically engineered mammalian cells in a NASA-designed rotating wall bioreactor. Rat 2lambda fibroblasts, genetically engineered to contain high-density (60 copies/cell) target genes for mutagenesis, were co-cultured with human epithelial cells on macroporous Cultispher-S beads. Light microscopy and histology were used to confirm cell attachment, distribution, and viability over the 8-day culture period. Key cell culture parameters (glucose, pH, and lactate) were monitored daily. Cells attached and completely covered the bead surface including the inner channels by day 4. Treatment of 4-day samples with dispase II dissolved the cultisphere and produced stable, bead-less spheroids. The spheroids were multi-cellular, had a well-organized extracellular matrix, and retained cell viability. The results suggest that stable multicellular spheroid models of uniform size can be produced in NASA bioreactors with genetically engineered cells for Earth-based studies as well as quantifying the potential health hazards attributed to the space environment. (Supported by NASA NRA-98-HEDS-02.).

VT-2003

The Effects of Exogenous Hormones on the Cytotoxicity of Chemically Modified Tetracyclines on LNCaP Human Prostate Tumor Cells. H.L. SAWKA, S.R. Simon and E.J. Roemer. Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794-8691. E-mail: hsawka@ic.sunysb.edu

The invasive and metastatic potential of prostate tumor cells may be linked to expression of one or more members of the family of matrix metalloproteinases (MMPs). We have been studying a number of chemically modified tetracyclines (CMTs), which lack anti-microbial properties, and are known to inhibit matrix metalloproteinases. Using LNCaP human prostate tumor cells in a variety of *in vitro* assays we have been evaluating which of these CMTs are the best candidates for development as potential prostate cancer treatments. The LNCaP cell line is an especially useful model because the cells retain some of the hormone sensitivity often seen in certain stages of prostate cancer *in vivo*. Phenol red (PR), used as a pH indicator in many media formulations, has hormone-like activity. By combining phenol red-free basal medium with charcoal dextran-stripped serum, we can essentially remove all exogenous hormones from the cells' culture environment. The cytotoxic effects of CMTs on prostate tumor cells in the absence and presence exogenous hormones were compared by growing cells in hormonally stripped experimental medium and in control medium containing phenol red and Fetal Bovine Serum (FBS). LNCaP cells were plated into 96 well culture plates at a density of 11.2×10^4 cells per well and incubated for 48 hours in normal growth (RPMI, 4%FBS) medium. The cells are then fed, with half of each plate receiving LNCaP normal growth medium (+PR), and half experimental hormone-depleted (phenol red-free RPMI, 4% charcoal-dextran stripped FBS) medium (-PR). The plates are incubated for an additional 48 hours and then the cells are dosed with 0, 10, 20, 30, 40 and 50 uM CMT. After a final 48 hour incubation in the presence of the drugs, MTS reagent is added to the wells, incubated for 4 hours, and the plates are read on an optical density plate reader set at 490um.. Data are graphed and an IC50 is calculated for each CMT based upon the values obtained from untreated control cells. The results showed that when a given CMT was cytotoxic in the dose range tested, the IC 50 was significantly lower in the presence of phenol red and regular serum then in the absence of phenol red and hormone containing serum. Additional experiments tested the direct effects of testosterone on LNCaP cells exposed to CMTs. Cells were grown for 4 days in the hormone depleted medium described above, supplemented with 0, 0.1, and 10 nM DHT. CMT exposure and MTS viability assay were performed as described. Results with DHT supplementation showed that when a CMT was cytotoxic in the dose range tested, the IC 50 decreased as the amount of DHT increased. [supported by NIH(NIDER) DE-10985; CollaGenex Pharmaceuticals, Inc.; USAMRMC DAMD-1798-18560; SUNYSB Center for Biotechnology (NYS Science & Technology Foundation) & NSF RAIRE# STI 9620074].

VT-2004

Cytotoxic Effects of Raloxifene on Mouse and Human Cancer Cell Lines. S.K. MAJUMDAR, M.C. Davis, and K. Ouchi. Department of Biology, Lafayette College, Easton, PA 18042. E-mail: majumdas@lafayette.edu

The toxicological potential of Raloxifene, a promising antibreast cancer drug was determined by using various assay techniques in murine erythroleukemic cells (MEL BB-88) and human cervical carcinoma cells (HeLa). Cell multiplication studies indicated that there was significant cell growth inhibition on BB-88 and HeLa cells by 5μg/ml Raloxifene and higher after 72 hours of treatment. The Neutral Red Toxicity Assay (Sigma) detected toxic effects of the agent on both BB-88 and HeLa cells within 24 hours at 5μg/ml treatment. The DePsipher Mitochondrial Membrane Potential Disruption Assay (Trevigen) revealed that the mitochondrial membrane potential in BB-88 and HeLa cells was altered by 10μg/ml Raloxifene at 48 hours and 24 hours, respectively. A disruption in the mitochondrial membrane potential is one of the first indicators of apoptosis induction. Raloxifene initiated nucleosomal fragmentation, another hallmark of apoptosis, at 24 hours of 10μg/ml treatment in both BB-88 and HeLa, as determined by Nucleosomal ELISA (Oncogene). Results from a surface ultrastructure morphological study depicted morphological changes, such as blebs and lamellipodia retraction in Raloxifene treated BB-88 and HeLa cells at 24 hours. The progression of alterations, such as cytoplasmic extrusions and holes leading to the complete rupturing of cell membranes, were observed with increased dosages of the drug and treatment time.

VT-2005

Assessing Tissue Specific Toxicity of Chemopreventive Agents in Cultures from Normal Human Tissues. E. ELMORE[1], T.-T. Luc[1] , G.J. Kelloff[2], V.E. Steele[2], and J.L. Redpath[1]. [1]Department of Radiation Oncology, University of California Irvine, Irvine, CA 92697; [2]Division of Cancer Prevention, NCI, NIH, Bethesda, MD 20892-7332. E-mail: eelmore@uci.edu.

The eight different epithelial cell lines or primary epithelial cell cultures (skin keratinocytes, renal cells, mammary cells, bronchial cells, cervical cells, prostate cells, oral mucosal cells, and hepatocytes were used to determine the comparative toxicity for twenty potential chemopreventive agents in the Human Epithelial Cell Cytotoxicity (HECC) Assay. The endpoints assessed were three and five day growth inhibition, mitochondrial function inhibition, and inhibition of proliferating cell nuclear antigen (PCNA) or albumin expression. Aspirin, s-allylcysteine, curcumin, DFMO, DHEA analogue 8543, L-selenomethionine, ursodiol, and vitamin E acetate were not toxic or only produced mild toxicity with all endpoints in all eight cell types. Agents that produced log differences (or greater) in sensitivity in one or more cell types were: N-acetyl-L-cysteine, calcium chloride, DHEA, genistein, ibuprofen, indole-3-carbinol, 4-HPR, oltipraz, piroxicam, PEITC, and P-xylylselenocyanate. For some agents such as DHEA, there was increasing toxicity (log differences in TC_{50} (toxic concentration that inhibited growth by 50%)) after five days compared to three days. Unique tissue-specific toxicity was observed for each toxic agent suggesting that tissue-specific effects are the rule rather than the exception. The HECC Assay is effective in identifying tissue specific toxicity for chemopreventive agents and should be effective for other types of agents.

VT-2006

Evaluation of Cell Viability during Cryopreservation using Cell Culture Medium versus Low-Temperature Storage Solutions. L.H. CAMPBELL, M.J. Taylor, and K.G.M. Brockbank. Organ Recovery Systems, Inc., Port City Center, Charleston, SC 29403. E-mail: lcampbell@organ-recovery.com

It is generally assumed that conventional culture media used to nurture cells at physiological temperatures will also provide a suitable medium for exposure at low temperatures. However, a comparison of cell culture medium with solutions designed to maintain the ionic and hydraulic balance in cells during exposure at low temperatures and/or freezing conditions has not been studied extensively. In this study, we compared cell viability after cryopreservation with either dimethyl sulfoxide (DMSO) or 1,2-propanediol (PD) in the base solutions, Dulbecco's Modified Eagle's Medium (DMEM), Euro-Collins (EC) or Unisol(tm) cryoprotectant vehicle (UHK-CV). Unisol(tm) is a new hyperkalaemic intracellular-type preservation solution designed to protect cells during hypothermic exposure. The addition of fetal calf serum (FCS) in these solutions was also evaluated to determine if the presence of FCS provided any benefit for recovery after freezing and thawing. Two cell lines were evaluated, a vascular smooth muscle cell line (A10) and a vascular endothelial cell line (CPAE). Cells were plated at 20,000 cells/well in 96-well microtiter plates (Falcon). Cryoprotectants (CPAs) were added using mannitol as a non-penetrating osmotic buffer and than the plates were cooled and stored overnight at -135 °C using a controlled-rate freezer (Planar). The next day, the plates were thawed using a two-step warming protocol and the CPAs were removed using 0.5M mannitol in regular cell culture medium. Metabolic activity after a suitable recovery period at 37 °C was assessed using Alamar Blue. Cell number was evaluated by measuring the DNA content of the wells using a fluorescent marker for nucleic acids (Cyquant, Molecular Probes). Two combinations of CPA and vehicle solution, DMSO/UHK-CV and PD/EC, demonstrated 75% cell viability or better with either cell type. Addition of FCS provided some additional benefit using the various combinations of CPA and base solution. Overall, EC and UHK-CV performed better than DMEM demonstrating improved viabilities with either CPA. In conclusion, optimum preservation of cells during low temperature storage is impacted by the nature of the base solution and its combination with cryoprotectants.

VT-2007

Enhanced Hypothermic Preservation of Human Renal Cells and Human Epidermal Keratinocytes. A.J. MATHEW, J.G. Baust, and R.G. Van Buskirk. Department of Biological Sciences and Institute of Biomedical Technology, State University of New York, Vestal Parkway East, Binghamton, NY 13902-6000; and BioLife Solutions, Inc., Mountain View Office Park, 820 Bear Tavern Road, Suite 106, Ewing, NJ 08628.

Hypothermic preservation reduces cellular metabolism thereby supporting the maintenance of cells, tissues, and organs for extended periods of time. Hypothermic solutions are now being tested as transport/storage media for cell therapy, engineered tissue, and organ transport applications. The period of cold storage is limited by the ability of a maintenance solution to prevent cellular damage and cell death resulting from profound hypothermia. Renal Proximal Tubule Epithelial Cells (RPTEC) and Normal Human Epidermal Keratinocytes (NHEK) were stored at 4°C in the hypothermic preservation solution, HypoThermosol (HTS), or HTS variants. RPTEC and NHEK that were stored in the variant solution HTS-FRS exhibited the highest levels of viability following cold storage, as measured by the alamarBlue metabolic indicator. In addition, RPTEC and NHEK were stained with Annexin V and Propidium Iodide (PI). RPTEC cells stored for up to 3 days in HTS demonstrated increasing levels of death. In comparison, death of cells maintained in HTS-FRS remained 10%. NHEK cells stored in HTS or HTS-FRS had similar levels of cell death at 1 day of storage. After 3 days of storage, however, 45% fewer of the HTS-FRS stored cells were dead. The data show that HTS-FRS is an improved hypothermic preservation media for the maintenance of cell viability. It also appears to prevent or delay the onset of cell death, as indicated by staining with Annexin/PI.

VT-2008

Comparison of Cell Viability Using Unisol and Other Preservation Solutions During Hypothermic Storage. L.H. CAMPBELL, M.J. Taylor, and K.G.M. Brockbank. Organ Recovery Systems, Inc., Port City Center, Charleston, SC 29403. E-mail: lcampbell@organ-recovery.com

In vitro manipulation of cells and tissues for transplantation calls for effective methods of preservation and hypothermic storage has proved to be the method of choice for organs. In the emerging field of tissue engineering, there is an urgent need to optimize techniques for the storage and shipping of component cells as well as for the final engineered product. Hypothermia involves cooling the organ to sub-physiological temperatures that suppresses metabolism and in essence, places the organ in a state of "suspended animation." Various cold storage solutions are currently available that can control or manipulate the environment in which the organ or tissue is being stored. In this way, protocols can be established that optimize the environment of any given cell, tissue or organ. We have developed a new hyperkalemic intracellular-type solution called Unisol(tm) (UHK) that has been formulated for hypothermic storage. We evaluated its effectiveness using three different cell types including a smooth muscle cell line (A10), an endothelial cell line (CPAE), and a kidney cell line (MDCK). These cells were plated in 96-well plates and stored at 4 °C for 1, 3, 5, and 7 days using Unisol and other established and currently available preservation solutions. These included Viaspan, Belzer Machine Perfusion Solution and Euro-Collins (EC). Control batches of cells were stored in regular cell culture medium, Dulbecco's Modified Eagle's Medium (DMEM), under similar conditions. Cell viability was assessed using the metabolic indicator Alamar Blue either immediately after hypothermic storage or for 7 consecutive days post-storage recovery at 37°C. After 1 day at 4°C, viability of kidney cells stored in UHK was >75% as compared to only 60% for Viaspan. Endothelial cells and smooth muscle cells also demonstrated a preference for UHK over Viaspan. The viability of endothelial cells in UHK was 70% versus only 55% for Viaspan while smooth muscle cells viability was 40% in UHK and only 10% in Viaspan. While survival is important, the ability of cells to recover and proliferate is equally important. Measurements of post-storage recovery demonstrated that cells were proliferating. For all three cell types, those stored in UHK appeared to recover faster and were able to proliferate more quickly than any of the other solutions. Thus, Unisol(tm) was shown to be a promising new hypothermic storage solution that warrants further evaluation for in vitro preservation of both natural and engineered tissues.

VT-2009

Withdrawn by author

VT-2010

Interleukin-8 (IL-8) as a Biomarker for Vesicant Agent-induced Cytotoxicity in Normal and Immortalized Human Keratinocytes. R. Vazquez, M. R. Nelson, J. J. Guzman, C. M. Corun, M. Steinberg[1], and C. M. Arroyo. Drug Assessment Division, U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400; [1]Biochemistry Division, The City College of the City University of New York, New York, NY 10031. Email: Raymond.Vazquez@Amedd.Army.mil

The recent availability of commercial immune assays for detection of inflammatory mediators has now made it possible to measure kinetics of the secretion of soluble inflammatory mediators in cell culture media. This study outlines the development of interleukin-8 (IL-8) as a biomarker for the detection of cytotoxic levels of sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) and Lewisite (2 chlorovinyldichloroarsine, L) in normal and immortalized (integrated origin/promoter segment of SV-40) human epidermal keratinocyte (HEK) cells. Primary normal and immortalized HEK cell lines have been grown at different cell densities and different cell passages. The immortalized HEKs resemble their primary cell counterparts, but have the advantage of being carried through long-term culture. Normal HEKs exposed to HD secreted pro-inflammatory mediators. We have postulated that pro-inflammatory mediators such as interleukin-1 beta (IL-b), IL-6, tumor necrosis factor-alpha (TNF-a) and IL-8 could be used as biomarkers of cutaneous vesicant injury. Immunoassay studies were performed to examine the response of these two cell lines to HD or L exposure. We found that normal HEKs secreted all the mentioned pro-inflammatory mediators when exposed to HD (100 mM) for 24 h. However, when normal HEKs were exposed to L (25-200 mM; 24 hours post-exposure time) only an upregulation of IL-8 levels was observed. The SV40-immortalized cells exposed to HD have a similar response, an upregulation of IL-1b, IL-6, TNF-a and IL-8. Lewisite exposure of SV40-immortalized HEKs blocked the response of IL-1b, IL-6 and TNF-a. In spite of that, a significant amount of IL-8 was secreted by SV40-immortalized HEKs. The present observation indicates that increased secretion of IL-8 by normal and SV40-immortalized HEKs represents an early event of the inflammatory reactions following L, and it is a good biomarker for L exposure. Future studies using state-of-the art *in vitro* biomagnetic resonance (NMR) techniques will be used to elucidate the pathophysiological pathway for L at low-doses of this chemical agent.

VT-2011

Human Epidermal Keratinocytes Exposed In Vitro to the Vesicating Agent Sulfur Mustard Express Markers of Apoptosis and Inflammation. W.J. SMITH, E.W. Nealley, O.E. Clark, and F.M. Cowan. Pharmacology Division, US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5400. Email: WILLIAM.SMITH@AMEDD.ARMY.MIL

Sulfur mustard (SM) is a chemical threat agent that causes blisters (vesication) when applied to the skin. It is a potent bifunctional alkylating agent capable of reacting with numerous nucleophilic targets in cells and tissues. Our laboratory is studying the mechanisms by which this compound creates its cutaneous pathology so that we can develop medical countermeasures to protect against the SM lesion. The primary tissue targets of SM in the skin are the basal epidermal cells. These are represented in culture by proliferating human epidermal keratinocytes (HEK). We have previously demonstrated that IL-8 was the one cytokine reproducibly expressed in culture by SM-exposed HEK (SIVB, 2000). Using flow cytometry, we have now seen that both IL-1α and IL-8 are expressed following exposures to 50-300M SM, whereas other cytokines, such as TNF-α, IL-1β and IL-6, show highly variable responses. These data, along with data presented last year at SIVB on Fc receptor induction and increased binding of C1q in SM-exposed HEK, suggest that SM-toxicity in HEK generates a cell capable of precipitating an inflammatory response that is a key component of the tissue pathology. Subsequently, we found that several pharmacological classes of therapeutic compounds can block the expression of IL-8 following SM. Our studies of the mechanisms of SM-induced cytotoxicity demonstrate that, while necrosis appears to be the predominant toxic manifestation, morphologic and biochemical characteristics of apoptosis, such as induction of caspase-3, can be detected. These data indicate that the toxic sequelae of exposure to a highly reactive compound such as SM, while multivariate, consist of discrete pathologies that can serve as targets for medical countermeasures.

VT-2012

The Identification and Quantification of Z-DNA in Congenital Cataracts. P. WANG+, C.E. Gagna+0, C. Philip+, and W.C. Lambert0. +Dept. of Life Sciences, New York Institute of Technology, Old Westbury, NY 11568; and 0Dept. of Pathology, University of Medicine and Dentistry-Medical School, Newark, NJ 07103.

The ocular lens of the eye globe is one of the few tissues that is transparent. This optical clarity is regulated by the arrangement of proteins called crystallins. When packing of crystallins is not correct cataract develops. Our group decided to use mice with congenital cataracts in order to characterize components of its cellular nucleic acids: left-handed Z-DNA. Mice eye globes (3 months) were fixed in a novel formalin-alcohol fixative. Two anti-Z-DNA polyclonal IgG antibodies were employed. Using a computerized image analysis system (200X) we mapped the distribution of cataractous lens secondary fibers (nucleated) and intensity of Z-DNA immunoreactivity. The congenital pathology was a subcapsular cataract. Immunohistochemistry of left-handed DNA revealed an increase in Z-DNA as compared to the control slides and non-cataractous secondary fiber cells. We speculate that the mutated (nucleated) secondary fiber cells (cataract) are undergoing an uncontrolled growth, in which Z-DNA, possibly a transcriptional enhancer, may be playing a major regulatory role. Z-DNA may act as a regulator of gene expression in normal cells. Crystallin genes within the ocular lens we used, may be defective, which are producing abnormal crystallins (proteins), thus allowing for cataractogenesis. These genes may have a much larger percentage of Z-DNA sequences. Our novel fixative allowed for superior examination of Z-DNA. Understanding the interaction of Z-DNA sequences and Z-DNA binding proteins may help to develop a method for diagnosing and curing human cataracts.

VT-2013

Characterization of a Human Conjunctival Epithelial Cell Line. Y.D. DIEBOLD, M. Calonge, R.M. Corrales, A. Enríquez de Salamanca, M.V. Sáez, and E. Pestaña. IOBA, University of Valladolid, Ramón y Cajal 7, E-47005 Valladolid, SPAIN. Email: yol@ioba.med.uva.es

Conjunctival epithelium contributes to the health of the ocular surface by producing and secreting components (mucins) of the tear film. Many pathological conditions of the conjunctiva impair this function. Therefore, it would be desirable to have an *in vitro* system to study the physiopathology of the human conjunctiva. A spontaneous cell line derived from a normal human conjunctiva (NHC) biopsy was characterized by our group. Cells were cultured in DMEM/F-12 supplemented with antibiotics, amphotericin B, insulin, EGF, cholera toxin, fetal bovine serum and hydrocortisone. Cell viability, plating efficiency, colony-forming efficiency and colony size were calculated in several passages. Different techniques were used to characterize NHC cells: chromosome analysis; Giemsa staining to observe general morphology; PAS and Alcian blue stainings to identify secretory cells and secretion products; transmission and scanning electron microscopy to confirm epithelial characteristics; immunofluorescence by using monoclonal antibodies against KI67, citokeratins, desmoplakins, vimentin, EGF receptor, FVIII, CD1 and AS02 to rate proliferative capability, to detect typical epithelial markers and to exclude contamination by other cell types; and RT-PCR analysis of mRNA expression for several mucin genes. Summarized, results showed that 1) cells had been continuously proliferating until passage 90, 2) contaminating cell types were absent, 3) epithelial characteristics are maintained *in vitro*, and 4) several mucin genes were expressed *in vitro*. We conclude that NHC cells retain many of the morphologic and functional characteristics of conjunctival epithelial cells *in vivo* and propose this cell line as a new *in vitro* system to study the physiopathology of the human conjunctiva.

VT-2014

Growth of Human Corneal Epithelial and Stromal Fibroblast Cells in Serum-Free Media. S.F.WEBB. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, ENGLAND. Email: s.f.webb@uea.ac.uk

Human corneal cell cultures are already successfully generated in our laboratories from un-transplantable corneo-scleral discs of all donor ages. Such corneas have been kept at the eye bank, often for more than 30 days and are hitherto, a rarely-tapped source for cell culture. The corneal layers are separated for explant culture by precise selective sectioning with a novel mini-microtome. Pure epithelial and fibroblast cells are grown routinely in EMEM containing 20% FCS. These cells will be used to construct miniature corneas for growth and toxicity studies. Although effective for cell growth, the constituents in FCS are neither all identifiable nor quantifiable. It is therefore preferable to use defined serum-free (SF) medium where the constituents can be modified. The purpose of this study is to find effective SF media for routine growth of human corneal cells. Five SF media (Clonetics) were tested against the control EMEM+FCS. Each medium was tested 10 times on primary cultures in 6-well plates. Four-day growth experiments (n) on first passaged (P) cultures were performed in 96-well plates. Cell growth was estimated by measurement of protein content using an absorbance colorimetric method. All five SF media sustained the initiation of culture but there was variation in the time needed for confluency. In three of them, this took over 3 weeks compared with 2 weeks in control EMEM+FCS but the remaining two SF media were twice as effective in promoting growth. The P cultures in four of the SF media consistently showed a faster rate of growth than in EMEM+FCS and two of these displayed double that of EMEM+FCS. It has thus been established that human epithelial and fibroblast cells can be successfully grown in defined SF media. Moreover these are corneas from donors ranging from 3-90 years old.

VT-2015

Cryopreservation of Immature Bovine Oocytes Treated with EGTA. L. SIMONETTI and M.R. Blanco. Facultad de Ciencias Agrarias. Universidad Nacional de Lomas de Zamora. Ruta 4 Km. 2 (1836), Llavallol, Buenos Aires, ARGENTINA. Email: L_SIMONETTI@HOTMAIL.COM

Previous results on immature bovine oocytes showed that exposition to EGTA at room temperature did not improve detrimental effects of cryopreservation procedures. The aim of this study was to asses morphological survival and *in vitro* maturation (IVM) of immature bovine oocytes exposed to EGTA at 39°C prior to cryopreservation. A total of 541 oocytes were obtained from 2-8 mm size of follicles of abattoir-recovered ovaries and assigned to groups: I (Control), II (EGTA+) and III (EGTA-)). Treatment with EGTA (1mM) in holding medium (ovum culture medium supplemented with BSA) was performed at 39°C for 5 min before CPA exposition. CPA consisting of 1.5 M ethyleneglycol (EG) in holding medium was added in three steps at 24°C for 15 min and oocytes were conventionally cryopreserved. After thawing at 37°C, CPA were removed and oocytes were cultured for IVM in TCM199 plus FBS, sodium piruvate, FSH, 17beta estradiol, hCG and antibiotics for 22 to 24 h at 39°C in 5% CO_2 in air. Then, cumulus cells were removed and morphology was assessed by stereoscopic examination. Normal morphology (NM) of oocytes was defined by observation of dark evenly granulated cytoplasm. Oocytes having NM were fixed, stained and evaluated for the presence of a first polar body and a metaphase II plate as signs of IVM. Data of morphology were analyzed by ANOVA and expressed as percentages (mean±SEM). Data of IVM were analyzed by Fisher's Exact test. NM for group I (89.1±4.1) was > II (37.8±8.4) and III (27.3±5.0) ($P<0.05$). IVM for group I (125/130) was > II (45/54) and III (34/43) ($P<0.05$). No differences were found between II and III neither for NM nor IVM ($P>0.05$). In conclusion, treatment with 1mM EGTA at 39°C seemed not to improve cryopreservation at least under our conditions.

VT-2016

3D Distribution of erb-B1 Receptors on Rat Colonocytes in Primary Cultures. BERTRAND KAEFFER[1], Alain Trubuil[2], Charles Kervrann[2], Marie-Françoise Devaux[3], and Christine Cherbut[1]. [1]Institut National Recherche Agronomique, Unité Fonctions Digestives et Nutrition Humaine, [2]Laboratoire de Biométrie INRA Jouy-en-Josas, [3]Unité de Recherche sur Microstructure et Macrostructure INRA Nantes , and INRA, UFDNH, BP 71627, 44316 Nantes Cedex 03 FRANCE. Email: kaeffer@nantes.inra.fr

Epithelial cells in intestinal tissues are building tubular structures maintained in a dynamic steady state by cells in proliferation-differentiation-apoptosis. These cells constitute a system wired by gap junctions allowing the rapid exchange of intracellular molecules and driven by extracellular informative molecules among which are the peptides of the epidermal growth factor family and their erb-B1 receptors. We have investigated the variation of erb-B1 receptors density on the surface of rat colonocytes by fluorescent laser scanning confocal microscopy performed onto living and fixed tissues maintained under microgravity in primary culture or on colonic crypts isolated by chelation or microdissection. Surface localization of EGF-Biotin and EGF-Alexa Fluor 488 complex binding onto erb-B1 receptors were checked by partial colocalization with connexin-32 structural protein of gap-junctions. Endodermic structural proliferative units from primary cultures cultured in rotating bioreactor for 5 to 40 days were found to contain 10 to 20 cells arranged according to a tubular symetry. EGF peptide binding was demonstrated in this structure along with gap junction structural protein by a recently designed 3-dimensional analysis software (Quant3D, Linux / Unix; sample images available from kaeffer@nantes.inra.fr). The model is available to test the current hypothesis about the diffusion of an unknown growth factor between adjacent cells by the network of epithelial cell gap junctions.

VT-2017

Preliminary Ultrastructural Data on the Innervation of the Interstitial Cells During the Differentiation of the Chick Ovary Cultured with LH or hCG. R.E. AVILA, M.E. Samar, R. Ferraris, F.J. Esteban*, J.A. Pedrosa*, and M.A. Peinado*. Dept. Histology, Embryology and Genetics. Faculty of Medical Sciences, National University of Cordoba. SeCyT (5000) Cordoba. ARGENTINA. *Dept. Cell Biology. School of Experimental Sciences. University of Jaén. E-23071, Jaén. SPAIN. E-mail: ravila@cmefcm.uncor.edu

In a previous work we demonstrated the relationship between nerve fibers and nerve endings and interstitial cells (steroid-producing cells) from the atrophic right ovary and the medulla in the left functioning ovary during embryogenesis in the chick, *in ovo*. Also, we showed the influence of the LH and hCG on the interstitial cells from chick embryo ovaries *in vitro*. There is growing evidence that ovarian steroidogenesis is controlled not only by pituitary gonadotropins but also by ovarian nerves. Besides, the local production of neurotrophins by steroidogenic cells is probably involved in the control of ovarian innervation. The objective of the present study was to analyze ultrastructurally the innervation during the differentiation of chick ovary cultured with LH or hCG. Explants of right and left ovaries from seven to nineteen days *in ovo* developed were cultured separately for 4 days in MEM in the presence of LH or hCG (problems) or 10% BSA (controls). Electron microscopic examination of the inervation explants from chick embryo ovaries revealed that the interstitial cells well innervated. Nerve fibres and nerve endings were observed in close contact with steroid-producing cells. Controls cultured showed a similar pattern of innervations that those of the same age *in ovo*. Problems cultured from seven days showed more conspicuous nerve endings than controls near the interstitial cells. These results *in vitro* suggest that innervation of the ovaries is controled by indirect mechanism via the hypothalamic-pituitary system and local production factors. More experiments are necessary to confirm these results.

VT-2018

The Effect of Protease Inhibitors on Triglyceride Synthesis and Insulin Signaling in L6 Myotubes. R.J. GERMINARIO and S.P. Colby-Germinario. Lady Davis Institute, SMBD Jewish General Hospital, McGill University Department of Medicine, Montrael, Quebec H3T 1E2, CANADA. Email: rgermi@po-box.mcgill.ca

Protease inhibitors (PI) as part of the combination drug therapies employed to treat AIDS patients result in side effects which include lipodystrophy and insulin resistance. We have investigated the effects of several PI on triglyceride synthesis (TG) and insulin signaling in the L6 myotube system. The various PI employed included Saquinavir (S), Ritonavir (R), and Indinavir (I). Insulin Receptor Substrate (IRS-1) and phosphatidyl inositol 3 kinase (PI-3K) were investigated. Detection involved Western analysis using specific rabbit polyclonal antibodies and for visualization the Amersham ECL system was used. TG was monitored by measuring the incorporation of ^{3}H-oleate into the cellular tirglyceride fraction. S, R, and I increased TG synthesis in a concentration dependent manner, the ranges being within those seen *in vivo*, for therapeutic efficacy (0.1 - 10 uM). The average insulin:control TG stimulation ration was 1.37 +/- .07 while the ratios observed for S, R, and I were 2.87, 1.47, and 1.5, respectively. In assessing the effects of S, R, and I on IRS-1 levels, we observed increased cellular IRS-1 content ranging from 1.2 to 2.0 times that seen in control cells +/- insulin (67nM). PI-3K levels were decreased by exposure to insulin (67nM) and this decrease was not seen on exposure to S, R, and I from 0.1 to 10 uM. The data indicates that PI can stimulate TG synthesis. Further, it was seen that the PI cause perturbations in specific insulin signals. The data suggests that this latter relationship has some bearing on the insulin resistance seen *in vivo*.

Index

Index

IN VITRO ANIMAL
CELLULAR & DEVELOPMENTAL BIOLOGY

Journal of the Society for In Vitro Biology

Revised 2/00

INFORMATION FOR AUTHORS

In Vitro Cellular & Developmental Biology is a journal of the Society for In Vitro Biology. Original manuscripts reporting results of research in cellular, molecular, and developmental biology that employ or are relevant to organs, tissue, tumors, and cells in vitro will be considered for publication. The complete scope of the journal is embodied in the following matrix:

Biotechnology
Cell and Tissue Models
Cell Growth/Differentiation/Apoptosis
Cellular Pathology/Virology
Cytokines/Growth Factors/Adhesion Factors
Signal Transduction
Toxicology/Chemical Carcinogenesis
Product Applications

MANUSCRIPT SUBMISSION REQUIREMENTS

When submitting a manuscript for consideration, authors should select one of the eight matrix headings, which best covers the scope of the manuscript submitted, as the section of the journal in which the article should appear.

The journal publishes three types of manuscripts: Regular Papers, Letters to the Editor, and Invited Reviews. Regular Papers are not restricted in respect to length, although page charges for papers over nine typeset pages are at a higher rate. Letters to the Editor are expedited original, peer-reviewed scientific reports of results of general or practical interest to readers. Letters to the Editor are usually limited to three printed pages and no more than three illustrations. They are subject to editorial review and editing prior to final publication to comply with the *Letters to the Editor* format. For *Letter to the Editor* format, authors should refer to previous issues of the journal. Nonscientific Letters to the Editor are welcomed and will be considered for publication but are subject to editorial or peer review. Unsolicited Reviews on relevant topics will be considered; however, it is recommended that authors contact the Editor prior to preparation. Both unsolicited and Invited Reviews are subject to rigorous editorial review, often in consultation with multiple experts in the field.

Animal manuscripts should be sent to: Wallace L. McKeehan, Editor; *In Vitro Cellular & Developmental Biology*; Institute of Biosciences and Technology; Texas A&M University System Health Science Center; 2121 W Holcombe Boulevard, Houston, Texas 77030-3303; Phone: (713) 677-7524; Fax: (713) 677-7512; E-mail: invitro@ibt.tamu.edu. **Authors outside North America may send manuscripts directly to the Editorial Office in Houston, Texas, or to an International Corresponding Editor.** At time of original submission, authors are required to submit three unstapled hard copies, a word processing disk (see requirements), and a completed copyright transfer form. Authors are also required to submit contact information for three to six individuals outside their institutions who are qualified to referee the manuscript. E-mail addresses and/or fax numbers are preferred. Authors may provide names of individuals they wish to exclude from the review process.

Plant-related papers may be sent directly to the Plant Editor, Gregory C. Phillips, New Mexico State University, Department of Agronomy & Horticulture, MSC 3Q, Gerald Thomas Hall-Room 254, P.O. Box 30003, Las Cruces, New Mexico 88003-8003; phone 505-646-5113, fax 505-646-6041, E-mail grphilli@nmsu.edu.

Manuscripts must be in concise English, on standard-size paper, typewritten, double-spaced (including tables, references, and legends for figures), and with 1-inch (2.54-cm) margins. **Authors must submit the final revised version of the manuscript on computer disk together with three hard copies.** Manuscripts not submitted on disk will result in an additional charge of $50 to the author. These should be sent to the Editor's Office. Either 3-½ or 5-¼" disks are acceptable, and they may be single- or double-sided and low or high density. Word for Windows is the preferred program. IBM-compatible/PC disks are preferred. Indicate on the disk label the manuscript number, the name of the first author, the software (including the version), and the system used.

Submission of a manuscript implies that all authors have agreed to its submission and that the manuscript is neither under consideration for publication elsewhere nor has appeared previously in part or in whole. Manuscripts will be reviewed by experts in their field. Reviewer and editorial opinions will be anonymously communicated to authors. Revised manuscripts will retain the original date of receipt if the revised version is received by the Editorial Office within 3 months.

ARRANGEMENT

The first page should carry title, author's name, institution, condensed title for running head (not to exceed 35 letters and spaces), and full address of the author who will receive proofs and reprint orders. You **MUST** include your telephone number, fax number, and e-mail address. The second page should contain a summary, not to exceed 250 words. **The summary serves as an abstract for abstracting journals and must be followed by four to six key words for indexing purposes.** These words should not be contained in the title.

The manuscript should be assembled in the following order: Title page (including full title, title footnote, byline, and running title), Footnotes, Summary, Introduction, Materials and Methods, Results, Discussion, Acknowledgment, References, Tables, Figure Legends, and Figures. Combination of Results and Discussion is acceptable. *All pages should be numbered consecutively starting with the title page.* Footnotes should be used only in tables as superscript lowercase letters and placed at the bottom of the page containing the table. Usage of abbreviations should be minimized. Spell out the abbreviated term followed by the abbreviation in parentheses where it is first cited in the summary, the text, and the figure legends. Abbreviations should not be used in the title.

REFERENCES

References must conform to the style of the *Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers.* 6th ed. In-text references should be cited parenthetically in author–year format in chronological, then alphabetical, order. A comma precedes the year, and entries are separated with a semicolon: (Murray and Kirchner, 1989; Pardee, 1989; Dirick et al., 1991, 1993b; Sherr, 1996).

The References listing should be arranged in alphabetical order. A single author precedes the same first author with coauthors. Provide *Index Medicus* journal title abbreviations; provide inclusive pages of work cited; provide title in the original language (when reproducible in the English alphabet), and state whether it is translated. Authors bear full responsibility for the accuracy of all references. Authors should confirm all references on the final manuscript with original publications. Cite as references only papers that have been accepted for publication. Cite manuscripts in preparation, unpublished results, personal communications, etc., parenthetically in the text. Authors should verify personal communications with the supplier of the information. The recommended citation style for references to manuals, books and journals is shown in the following examples:

Council of biology editors style manual. CBE style manual committee, 5th ed. Bethesda, MD: Council of Biology Editors; 1983.

Mokul'skaya, T. D.; Smetanina, E. P.; Mychko, G. E., et al. Secondary structure of DNA from phages T_4 and T_6. Mol. Biol.

(Moscow) 9:445–449; 1976. Translation of Mol. Biol. (Moscow) 9:552–555; 1975.
Trowell, O. A. Tissue culture in radiobiology. In: Willmer, E. N. ed. Cells and tissues in culture. Methods, biology and physiology. Vol. 3. London: Academic Press; 1966:63–149.
Weinhold, P. A.; Burkel, W. E.; Fischer, T. V.; Kahn, R. H. Adult rat lung in organ culture: maintenance of histopathic structure and ability to synthesize phospholipid. In Vitro Cell Dev. Biol. 15:1023–1031; 1979.

ILLUSTRATIONS, FIGURES, AND TABLES

All photographs, text figures, and tables should be mounted on standard-size white paper, numbered in agreement with the text, identified with the title of the manuscript and the author's name, and placed after the last page of text with each page numbered. Each article is permitted two illustrations at no charge. **Illustrative material in excess of two illustrations will be charged to the author at an additional $15.00 per illustration. The cost of all color illustrations must be borne by the author and are payable in advance.** The cost of color printing is currently $1300 for the first page and $800 for each additional page. A pro forma invoice will be sent to the corresponding author.

Photographs should be printed on glossy paper. Photographs and figures will be reduced to one column width (3-⅜", 8.6 cm) unless the authors justify the use of additional space. Authors who wish to have any figures printed at greater than one-column width should obtain the approval of the managing editor. The magnification of photomicrographs should be stated.

Figure Legends should be collected at the end of the manuscript. Avoid lengthy legends with detailed experimental protocols. State experimental conditions pertaining to the illustrations but avoid repetition of the text. Explain all symbols and abbreviations used in the illustrations. Simple symbols such as ○, Δ and ■ should be used. The use of error bars on the data points is recommended. Indicate the number of the figure, the top of the drawing, and the author's name. Figures should be numbered consecutively with Arabic numerals.

If possible, please use bulleted or numbered lists within the text instead of tables. If tables are required, they should carry short titles and should be intelligible without reference to the text.

CELL LINE AND REAGENT DATA

The source of cells utilized, species, sex, strain, race, age of donor, and whether primary or established should be clearly indicated. **The name, city, and state or country of the source of reagents should be stated within parentheses when first cited.** Specific tests used for verification of cell lines and novel reagents should be identified. Specific tests for the presence of mycoplasmal contamination of cell lines are recommended. If these tests were not performed, this fact should be clearly stated. Other data relating to unique biological, biochemical, and/or immunological markers should also be included if available. Publication of results in *In Vitro Cellular & Developmental Biology* is based on the principle that results must be verifiable. Authors are expected to make unique reagents available to qualified investigators. Authors deriving or using cell lines are encouraged to follow the UKCCCR Guidelines for the Use of Cell Lines in Cancer Research in respect to validation of identity and infection-free cultures (http://ukcccr.icnet.uk/cell_lines_guides.html).

NOMENCLATURE

The recommendations of the Society for In Vitro Biology Committee on Terminology should be followed. Schaeffer, W. I. Terminology associated with cell, tissue and organ culture, molecular biology and molecular genetics. In Vitro Cell. Dev. Biol. 26:97–101; 1990.

COPYRIGHT

To maintain and protect the Society's ownership and rights and to protect the original authors from misappropriations of their work, SIVB requires the corresponding author to sign a copyright transfer agreement on behalf of all the authors. Unless this agreement is executed (without changes or addenda), SIVB will not publish the manuscript.

If *all* authors were employed by the U.S. government when the work was performed, the corresponding author should not sign the copyright transfer agreement but should instead attach to the agreement a statement attesting that the manuscript was prepared as a part of their official duties and, as such, is a work of the U.S. government not subject to copyright.

If *some* of the authors were employed by the U.S. government when the work was performed but the others were not, the corresponding author should sign the copyright transfer agreement as it applies to that portion performed by the nongovernment employee authors.

PAGE CHARGES

It is anticipated that page charges, currently $45.00 per page up to nine pages for regular papers and three pages for Letters to the Editor ($35.00 for SIVB members) and $100.00 for subsequent pages, will be paid by authors whose research was supported by special funds, grants (departmental, governmental, institutional, etc.) or contracts or whose research was done as part of their official duties. A pro forma invoice for page charges is sent with page proofs and reprint order form. The Society must have either a check or a copy of the purchase order for the full amount due prior to a paper being scheduled for publication.

If research was not supported by any of the means described above, a request to waive the charges should be sent to the Editor, with the disk of the manuscript text prior to the time of paper acceptance. This request, which must be separate from the cover letter, must indicate how the work was supported and should be accompanied by a copy of the Acknowledgment section.

ALTERATION CHARGES

It is necessary to charge for changes made to a paper after it has been set into type. A $6.00 charge for each change will be imposed and must be included on the pro forma invoice by the author when returning the page proof. This is a "pass through" charge from the compositor to the author. A check, copy of a purchase order, or authorization to charge a credit card should accompany author's pages and pro forma invoice when returned for publication.

Authors are responsible for the contents of their papers, including changes made by the copy editor. Authors will be charged for excessive changes in proofs.

REPRODUCTION OF PAPERS

IN VITRO
PLANT
CELLULAR & DEVELOPMENTAL BIOLOGY

INFORMATION FOR AUTHORS

In Vitro Cellular & Developmental Biology— PLANT is a bimonthly journal of the Society for In Vitro Biology (SIVB) and the International Association for Plant Tissue Culture & Biotechnology (IAPTC&B). Four regular SIVB issues (numbers 1, 3, 4 and 6; published January/February, May/June, July/August, November/December) include original manuscripts describing results of cellular, molecular and developmental biology research using *in vitro* grown or maintained organs, tissues or cells derived from plants. Studies that relate findings *in vitro* to plant biotechnology and genetics are especially welcomed to be considered for publication.

The complete scope of the journal is embodied in the following matrix:

1. **Biotechnology / Genetic Transformation / Functional Genomics** — Transformation methods; transgene expression/regulation; plant expression vectors; promoter/enhancer/scaffold attachment/transgene product structure-function; field evaluation.
2. **Cell Biology** — Cell cycle; cytodifferentiation; cell-cell interactions/signaling; protein targeting; organelles; structure-function.
3. **Developmental Biology / Morphogenesis** — Embryogenesis; embryogeny; gametogenesis; organogenesis; floral differentiation/development.
4. **Metabolic Engineering / Secondary Metabolism** — Biotransformation; metabolic flux; bioactive/medicinal compounds.
5. **Micropropagation** –- Acclimatization; automation; bioreactors; cloning; contamination control; disease indexing; scale-up.
6. **Molecular Farming** — Transgenic production of recombinant proteins and pharmaceuticals such as antibodies, edible vaccines, industrial enzymes, etc.
7. **Physiology** — Plant growth regulators; primary metabolism; regulatory processes; signal transduction.
8. **Somatic Cell Genetics** — Somaclonal variation; epigenetics; mutagenesis; somatic hybridization; T-DNA/transposon tagging; homologous recombination.

Two special issues (numbers 2 and 5, published March/April and September/October) are published as the official journal of the IAPTC&B, and include original manuscripts, feature and review articles, commentary, and IAPTC&B official notifications. The IAPTC&B maintains a completely separate and independent international editorial review board for these two issues. Original manuscripts dealing with plant tissue culture and molecular and cellular aspects of plant biotechnology are the focus of these two issues and are not divided into matrix categories.

MANUSCRIPT SUBMISSION REQUIREMENT

When submitting a manuscript for consideration, authors should select one of the eight matrix headings, which best covers the scope of the manuscript submitted, as the section of the journal in which the article should appear.

Invited reviews and letters-to-the-editor will also be published.

In Vitro Plant manuscripts should be sent to: Dr. Gregory Phillips, New Mexico State University, Dept. of Agronomy and Horticulture, Box 30003/MSC 3Q, Arid Lands Bldg. – Room N127, Las Cruces, NM 88003-8003, USA (Tel. 505-646-3297, Fax. 505-646-6041, Email: grphilli@nmsu.edu). At time of original submission, authors are required to submit four hard copies and a completed copyright transfer form.

In Vitro Animal manuscripts should be sent directly to: Wallace L. McKeehan, Editor, *In Vitro Cellular & Developmental Biology — Animal*, Albert B. Altek Institute of Biosciences and Technology, Texas A&M University, 2121 W. Holcombe Blvd., Houston, TX 77030-3303, USA (Tel. 713-677-7522).

The manuscripts for the special two IAPTC&B issues should be sent to either of the co-editors: Dr. Trevor Thorpe, University of Calgary, Department of Biological Sciences, 2500 University Drive, N.W. Calgary, Alberta, Canada T2N 1N4 or Dr. David Altman, ProfiGen, 800 Harrison St., Nashville, TN 37203, USA.

Articles should be timely and precisely presented. Manuscripts must be in concise English, on standard size paper, typewritten, double spaced (including tables, references, and legends for figures), and with 1-inch (2.54 cm) margins. **Authors must submit final revised version of manuscript on computer disk together with three hard copies.** Manuscripts not submitted on disk will result in an additional charge of $50 to the author. These should be sent to Dr. Phillips' office in Las Cruces, NM. $3\frac{1}{2}$" disks are preferred either single- or double-sided, low or high density. Microsoft Word 97 for Windows is the preferred program, but we can accept WordPerfect 5.1 for DOS and WordPerfect 6.1 for Windows. (If a version higher than listed here is used, supply an ASCII (DOS) text file.) IBM compatible/PC disk is preferred. Indicate on the disk label the manuscript number, the name of the first author, the software (including version), and the system used.

Submission of a manuscript implies that all authors have agreed to its submission and that the manuscript is neither under consideration for publication elsewhere nor has appeared previously in part or in whole. Manuscripts will be reviewed by two or three experts in the relevant field. Authors may suggest desired reviewers in their field. Reviewer and editorial opinions will be anonymously communicated to authors. Revised manuscripts will retain the original date of receipt if the revised version is received by the Editorial Office within 3 months.

ARRANGEMENT

Each section should start on a new page. The first page should carry: Title, Author's name, Institution, condensed title for running head (not to exceed 35 letters and spaces), and full address of the author who will receive proofs and reprint orders. The second page should contain a summary, not to exceed 250 words. **The summary serves as an abstract for abstracting journals, and must be followed by the listing of four to six key words for indexing purposes.** These words should not be contained in the title.

The manuscript should be assembled in the following order: title page (including full title, title footnote, byline, and running title), footnotes, SUMMARY, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENT, REFERENCES, TABLES, FIGURE LEGENDS, and FIGURES. Combination of RESULTS and DISCUSSION is acceptable. *All pages should be numbered consecutively starting with the title page.* Footnotes should be used only in tables as superscript lower case letters, and placed at the bottom of the page containing the table. All acknowledgments should be on a separate sheet preceding the references. Use of abbreviations should be minimized. Where necessary, spell out the abbreviated term followed by the abbreviation in parentheses where it is first cited both in the text and in the legends. Abbreviations should not be used in title or summary.

MATERIALS AND METHODS

Complete identification of suppliers, along with their city, state, and country, should be given for unique or unusual materials used in experiments. Methods should be clear and concise, but provide sufficient detail to allow others in the scientific community to interpret or verify the results.

REFERENCES

References must conform to the Name–Year system as described by the *Council of Biology Editors' Style Manual, 6th Edition.* In this system the references at the end of a document are arranged alphabetically by the name of the first author. Within the text the reference should be cited with the name and date: for example, "Organ cultures were initiated as previously described (Smith et al., 1989)" or "Organ cultures were described by Smith et al. (1989)". Follow *Index Medicus* journal title abbreviations; provide inclusive pages of work cited; provide title in the original language

(when reproducible in the English alphabet) and state if it is translated. Authors bear full responsibility for the accuracy of all references. Authors should confirm all references on the final manuscript with original publications. Cite as references only papers that have been accepted for publication. Cite "manuscripts in preparation," "unpublished results," "personal communications," etc., in the text. Authors should verify personal communications with the supplier of the information. The style of reference for books, journals, and chapters within books is as below:

George, E. F.; Sherrington, P. D. Plant propagation by tissue culture: handbook and directory of commercial laboratories. London: Exegetics Ltd.; 1984:102–110.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–479; 1962.

Thorpe, T. A. Organogenesis *in vitro*. In: Vasil, I. K., ed. Perspectives in plant cell and tissue culture. Int. Rev. Cytol., Suppl. 11A. New York: Academic Press; 1980:71–105.

ILLUSTRATIONS AND FIGURES

All photographs and line figures should be on standard size white paper, numbered in agreement with the text, identified on the reverse side with the title of the manuscript and the author's name and placed after the last page of text with each page numbered.

Photographs should be printed on glossy paper. Photographs and figures will be reduced to one column width (3-3/8", 8.6 cm) unless the authors justify the use of additional space. Authors who wish to have any figures printed at greater than one-column width should obtain the approval of the managing editor. The magnification of photomicrographs should be stated.

It is recommended to supply line figures on disk with the text. Preferred programs are Word and Excel for Windows, Adobe Illustrator and Adobe Photoshop. If using any program other than Word or Excel for Windows, the figure should be supplied as a TIF image. There is no charge if figures are not supplied on disk, but it will assist the production process and ensure a better quality of reproduction. It is not recommended to supply photographs on disk because of quality problems. Even if the figures are supplied on disk, is **essential** that a good quality hard copy print of every figure is supplied.

Figure legends should be collected at the end of the manuscript. Avoid lengthy legends with detailed experimental protocols. State experimental conditions pertaining to the illustrations but avoid repetition of the text. Explain all symbols and abbreviations used in the illustrations. Simple symbols such as ○, Δ, and ■ should be used. The use of error bars on the data points is recommended. On the back of each figure, indicate in soft pencil the number of the figure, the top of the drawing, and the author's name. Figures should be numbered consecutively with Arabic numerals and their approximate positions should be indicated in the margins of the text.

TABLES

If possible, please use bulleted or numbered lists within the text instead of tables. If tables are required, they should carry short titles, and should be intelligible without reference to the text. The tables should be included on the text disk.

ILLUSTRATION AND PAGE CHARGES*

There are no page charges up to nine typeset pages. Authors will incur a $100 charge for each page exceeding nine pages.

Each article is permitted the equivalent of one full page of tables, photographs, and figures at no cost. After the first page, illustration charges will be billed at the rate of $55.00 ($45.00 for SIVB members) per full page.

The cost of all color illustrations must be borne by the author and is payable in advance. The cost of color printing is currently $1300 for the first page and $800 for each additional page. A proforma invoice will be sent to the corresponding author.

It is anticipated that any applicable page and illustration charges will be paid by authors whose research was supported by special funds, grants (departmental, governmental, institutional, etc.) or contracts or whose research was done as part of their official duties. A proforma invoice for applicable page and illustration charges will be sent with page proofs and a reprint order form. The Society must have either a check, authorization to charge a credit card, or a copy of the purchase order for the full amount due prior to a paper being published.

If the research was not supported by any of the means described above, a request to waive the charges may be sent to Gregory Phillips, editor, with the disk of the manuscript text at the time of paper acceptance. This request, which must be separate from the covering letter, must indicate how the work was supported and should be accompanied by a copy of the Acknowledgment section.

*These charges are for the 2001 volume and may change for publication after 2001.

ALTERATION CHARGES

For the four regular SIVB issues (nos 1, 3, 4, 6), it is necessary to charge for changes made to a paper once it is at page proof stage. A $50.00 per page charge for each page on which there is a correction will be imposed and must be included on the proforma invoice by the author when returning the page proof. This is a "pass through" charge from the compositor to the author. A check, authorization to charge a credit card, or copy of a purchase order should accompany authors' pages and proforma invoice when returned for publication. *There is no charge for typographical errors – however any change from the original manuscript is deemed an author's alteration.* We are not charging author alterations to the IAPTC&B authors.

NOMENCLATURE

The recommendations of the Society for In Vitro Biology Committee on Terminology should be followed. Schaeffer, W. I. Terminology associated with cell, tissue and organ culture, molecular biology and molecular genetics. In Vitro Cell. Dev. Biol. 26:97–101; 1990.

COPYRIGHT

To maintain and protect the Society's ownership and rights and to protect the original authors from misappropriations of their work, SIVB requires the corresponding author to sign a copyright transfer agreement on behalf of all the authors. Unless this agreement is executed (without changes and/or addenda), SIVB will not publish the manuscript.

If *all* authors were employed by the U.S. government when the work was performed, the corresponding author should not sign the copyright transfer agreement but should, instead, attach to the agreement a statement attesting that the manuscript was prepared as a part of their official duties and, as such, is a work of the U.S. government not subject to copyright.

If *some* of the authors were employed by the U.S. government when the work was performed but the others were not, the corresponding author should sign the copyright transfer agreement as it applies to that portion performed by the non-government-employee authors.

REPRODUCTION OF PAPERS

Permission is granted to quote from *In Vitro Cellular & Developmental Biology — Plant* in scientific works with the customary acknowledgement of the source. The reprinting of a figure, table or excerpt requires the consent of one of the original authors and the notification of *In Vitro — Plant*. Reprinting beyond that permitted above requires written permission from the copyright holder, the Society for In Vitro Biology (SIVB), and payment of an appropriate royalty.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by the Society for In Vitro Biology for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of $10.00 per copy per article (no per page fee) is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923.

2001 Congress on In Vitro Biology

Exhibitor List

As of April 15, 2001

Ambion, Inc.
Austin, TX

AMS Midlands
Medical Technologies
Mediquip Parts Plus
St. Louis, MO

Bio-Rad Labs
Hercules, CA

BioPharm
Edison, NJ

BTX, Div. of Gentrn
San Diego, CA

CABI Publishing
Wallingford, Oxon, UK

Conviron
Boiling Springs, SC

Cook Biotech, Inc.
West Lafayette, IN

Guava Technologies
Burlingame, CA

Harvard Bioscience
Holliston, MA

Innovative Cell Technologies
San Diego, CA

InvitroGen - Life Technologies
Rockville, MD

Monsanto Company
St. Louis, MO

Olympus America, Inc.
Melville, NY

Percival Scientific, Inc.
Boone, IA

Phyto Technology Laboratories
Overland Park, KS

Promega Corporation
Madison, WI

Sigma-Aldrich Corporation
St. Louis, MO

ThermoForma Scientific
Marietta, OH

VWR Scientific Products
St. Louis, MO

Reception

EXHIBIT HALL MONDAY, JUNE 18, 12:30 - 1:15 PM

Co-Sponsored By:

Ambion, Inc.
Austin, TX

BTX, Div. of Gentrn
San Diego, CA

CABI Publishing
Wallingford, Oxon, UK

Conviron
Boiling Springs, SC

Cook Biotech, Inc.
West Lafayette, IN

Harvard Bioscience
Holliston, MA

Innovative Cell Technologies, Inc.
San Diego, CA

InvitroGen - Life Technologies
Rockville, MD

Monsanto Company
St. Louis, MO

Olympus America, Inc.
Melville, NY

Percival Scientific Inc.
Boone, IA

Phyto Technology Laboratories
Overland Park, KS

Promega Corporation
Madison, WI

Society for In Vitro Biology
Largo, MD

ThermoForma Scientific
Marietta, OH

Registration Bag Advertisers

As of April 2, 2001

ICN – Biomedical, Inc.
Costa Mesa, CA

Innovative Cell Technologies, Inc.
San Diego, CA

2001 Congress on In Vitro Biology

Acknowledgments

The Planning Committee acknowledges the contributions received from the following companies and organizations for their support of scientific and educational programs.

Congress Sponsors *(Contributions up to $25,000)*

Aventis CropScience
Monsanto Company (Congress Plenary Session Co-Sponsor)
UST, Inc.

Congress Symposia Sponsors *(Contributions up to $5,000)*

Amersham Pharmacia Biotech
Biowhittaker, Inc.
BTX, Division of Genetronics
Colgate Palmolive Company (Education Program Sponsor)
Defense Advanced Research Projects (DARPA)
DowAgro Sciences, LLC
DuPont Agricultural Products (Congress Plenary Session Co-Sponsor)
Eli Lilly and Company
John Wiley & Sons, Inc.
Life Technologies Incorporated, A Division of InvitroGen
Protein Technologies International
Renessen LLC
RiceTec Incorporated
Society for Cryobiology
Syngenta Crop Protection AG
The Scotts Company

Congress Contributors *(Contributions up to $1,500)*

ArborGen, LLC
Argonex Discovery Ltd., UK
ATCC
Avon Products, Inc.
Bennett & Company Ltd., UK
Bio-Rad Laboratories Incorporated
Bionique Testing Labs, Inc.
CN Bioscience Ltd., UK
ECACC, UK
Forma Scientific Incorporated
Garst Seed Company
International Foundation for Ethical Research
John Stanair and Company, UK
Johnson & Johnson
Merck Research Laboratories
Olympus, UK
Organ Recovery Systems Incorporated
PerkinElmer Life Science
Rohm Haas Company
ThermoForma Scientific

The International Association for Plant Tissue Culture & Biotechnology (IAPTC&B)

announces the

10th IAPTC&B Congress
Plant Biotechnology 2002 and Beyond

Disney's Coronado Springs Resort, Orlando, Florida
June 23-28, 2002

The 10th IAPTC&B Congress is being planned as a major international event. In partnership with academia and industry, it will showcase and celebrate the science, technology, and products of plant biotechnology. The 10th IAPTC&B Congress is organized by the U.S. and Canadian Chapters of the IAPTC&B, the Plant Section of the Society for In Vitro Biology, and the University of Florida. In addition to the above, early sponsors of the Congress include Danone Group, Epicyte Pharmaceutical, Exelixis Plant Sciences, Kluwer Academic Publishers, Monsanto, Nature Biotechnology, Nestlé, Novartis, Paradigm Genetics, Phytomedics, Pioneer, RiceTec Inc., The Plant Journal, The Scotts Company, U.S. National Aeronautics and Space Administration, UST/ProfiGen, and Walt Disney World.

About the Congress Program - An outstanding, world-class scientific program is being developed and will feature plenary lectures, symposia, workshops, and posters on the latest developments and issues in modern plant tissue culture and biotechnology. The plenary and symposia lectures will be published in the congress proceedings by Kluwer Academic Publishers.

Fellowships for Graduate Students and Post-doctoral Associates - The IAPTC&B will provide a limited number of fellowships for young scientists, students, post-docs, and participants from developing countries. Fellowships will be awarded based on the quality of submitted abstracts.

Exhibiting at the Congress - The Congress will host a Science and Technology Exhibit for industry and academic institutions to showcase their technologies, programs, products, and vision for the future. This is an opportunity for organizations to meet face-to-face with thousands of plant biotechnology researchers and professionals from around the world. This is the largest gathering of its type and meets only once every four years. It is an opportunity not to be missed!

IAPTC&B Officers

Indra K. Vasil (USA) - IAPTC&B President
(e-mail: ikv@mail.ifas.ufl.edu)

David W. Altman (USA) - IAPTC&B Secretary-Treasurer
(e-mail: 102133.3263@compuserve.com)

Important Deadlines

Fellowship Opportunities – November 1, 2001
Early-Bird Registration – January 15, 2002
Advance Registration – May 24, 2002
Hotel Registration – May 24, 2002

For registration, abstract, housing, and fellowship information, go to our web site at www.sivb.org

For further information about the IAPTC&B and the 10th IAPTC&B Congress, visit our web site: www.hos.ufl.edu/iaptcb

To receive future mailings for the 10th IAPTC&B Congress, please contact the IAPTC&B Congress:
% Society for In Vitro Biology
9315 Largo Drive, West, Suite 255
Largo, MD 20774 USA
Tel: (301) 324-5054 or 1-800-741-7476 (USA/Canada)
Fax: (301) 324-5057
E-mail: sivb@sivb.org

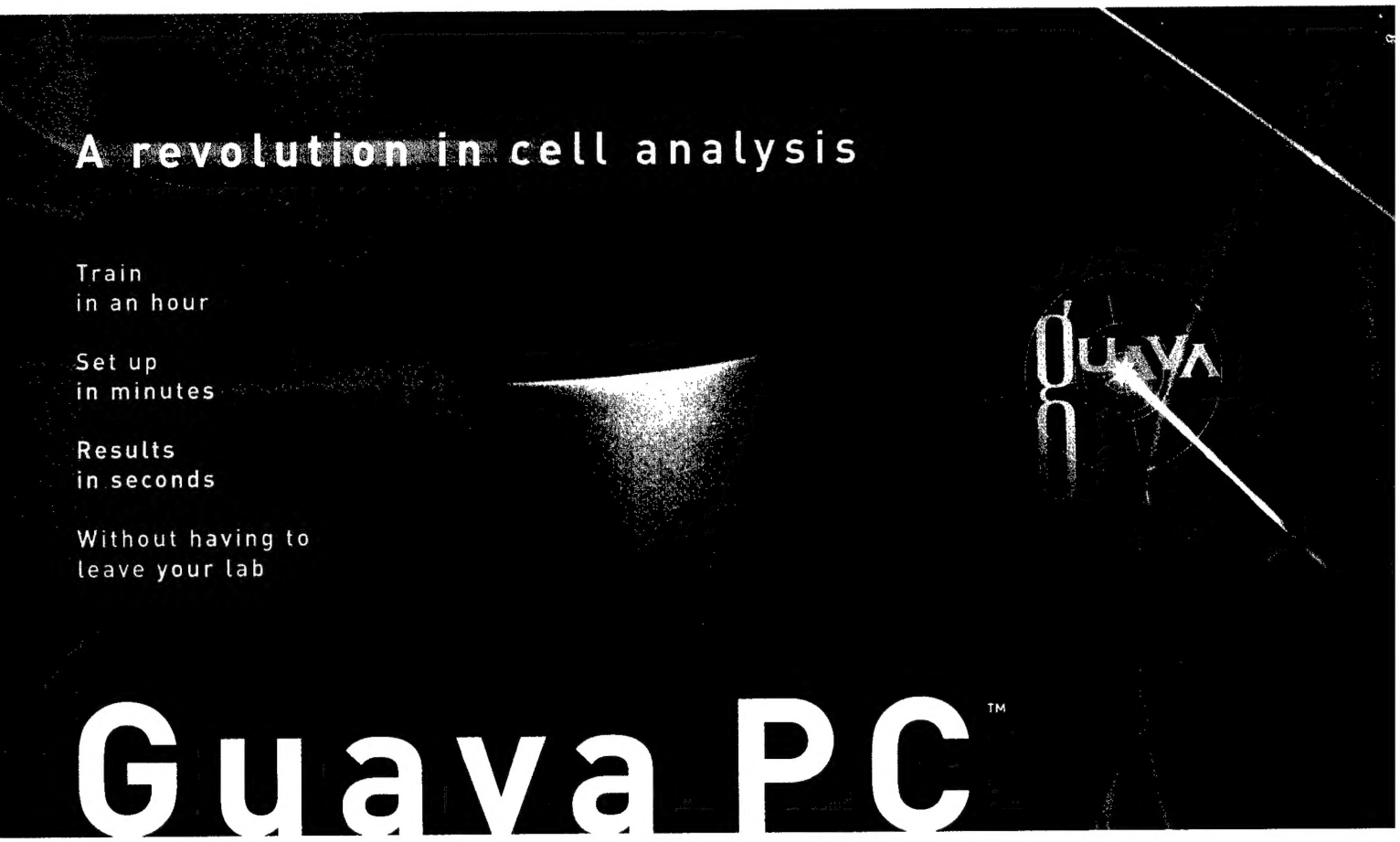
Plenary Speakers

David Baulcombe (UK), Normal Borlaug (USA), Steven Briggs (USA), Robb Fraley (USA), Mich Hein (USA), Dirk Inze (Belgium), Jonathan Jones (UK), Anna Koltunow (Australia), Maurice Moloney (Canada), Ilya Raskin (USA), Kazuo Shinozaki (Japan), Ron Sederoff (USA), Lothar Willmitzer (Germany), Marty Yanofsky (USA)

Symposia Speakers*

Klaus Ammann (Switzerland), Gynheung An (Republic of Korea), Charles Arntzen (USA), Howard Atkinson (UK), Vincent Chiang (USA), Nam-Hai Chua (USA), Sacco de Vries (The Netherlands), Walter DeWitte (UK), John Duvick (USA), David Ellar (UK), Dennis Gonsalves (USA), Bill Gordon-Kamm (USA), Jonathan Gressel (Israel), John Harada (USA), Erwin Heberle-Bors (Austria), Luis Herrera-Estrella (Mexico), T.J. Higgins (Australia), Tom Hoban (USA), Harry Klee (USA), Abe Krikorian (USA), Chin-Yi Lu (Australia), Julian Ma (UK), Rich Meagher (USA), Alejandro Mentaberry (Argentina), Brian Miki (Canada), David Ow (USA), Peggy Ozias-Akins (USA), Stephen Padgette (USA), Gerald Perbal (France), Fred Perlak (USA), Ingo Potrykus (Switzerland), Rafael Rivera-Bustamante (Mexico), John Ryals (USA), Goran Sandberg (Sweden), German Spangenberg (Australia), Brian Staskawicz (USA), Jennifer Thompson (South Africa), Zhi-Hong Xu (China), Usha Barwale Zehr (India)

*An additional 85-95 speakers will be selected from submitted abstracts.



Guava Technologies, Inc. introduces the first Personal Cytometer.

Small size. Small price. Highly convenient. With results equal or superior to instruments that cost several times more, this powerful automated system lets you perform many critical applications right at your bench.

Cell Count and Viability Assays
Protein Expression Assays
Apoptosis Assays
Expandable to future applications

No specialized training needed. No maintenance. No worries. It uses simple protocols and no sheath fluid, so preparation goes swiftly and cleaning is a snap. The Guava PC's proven turnkey system comes complete with validated reagents, solid-state laser precision, and automated data analysis. **If it were any easier, the cells would analyze themselves.**

To list all the Guava PC's novel features would take a whole day, but we could show you how it works in less than an hour.

Call 866.448.2827 or visit www.guavatechnologies.com to find out how the Guava PC will revolutionize your laboratory.

Visit us at SIVB Booth 34 to see the Guava PC and an applications demonstration. Ask about our introductory price offer and enter the contest for a free digital camera.

Guava technologies inc